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(54) Title: 12303, A NOVEL HUMAN TWIK MOLECULE AND USES THEREOF

(57) Abstract: The invention provides isolated nucleic acids molecules, designated TWIK-8 nucleic acid molecules, which encode novel TWIK-related potassium channel subunit molecules. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing TWIK-8 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a TWIK-8 gene has been introduced or disrupted. The invention still further provides isolated TWIK-8 proteins, fusion proteins, antigenic peptides and anti-TWIK-8 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.



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12303, A NOVEL HUMAN TWIK MOLECULE AND USES THEREOF**Related Applications**

This application claims priority to U.S. Provisional Patent Application No.
5 60/195,734, filed on April 7, 2000, incorporated herein in its entirety by reference.

Background of the Invention

Potassium (K^+) channels are ubiquitous proteins which are involved in the setting of the resting membrane potential as well as in the modulation of the electrical
10 activity of cells. In excitable cells, K^+ channels influence action potential waveforms, firing frequency, and neurotransmitter secretion (Rudy, B. (1988) *Neuroscience*, 25, 729-749; Hille, B. (1992) *Ionic Channels of Excitable Membranes*, 2nd Ed.). In non-excitable cells, they are involved in hormone secretion, cell volume regulation and potentially in cell proliferation and differentiation (Lewis *et al.* (1995) *Annu. Rev.*
15 *Immunol.*, 13, 623-653). Developments in electrophysiology have allowed the identification and the characterization of an astonishing variety of K^+ channels that differ in their biophysical properties, pharmacology, regulation and tissue distribution (Rudy, B. (1988) *Neuroscience*, 25, 729-749; Hille, B. (1992) *Ionic Channels of Excitable Membranes*, 2nd Ed.). More recently, cloning efforts have shed considerable
20 light on the mechanisms that determine this functional diversity. Furthermore, analyses of structure-function relationships have provided an important set of data concerning the molecular basis of the biophysical properties (selectivity, gating, assembly) and the pharmacological properties of cloned K^+ channels.

Functional diversity of K^+ channels arises mainly from the existence of a great
25 number of genes coding for pore-forming subunits, as well as for other associated regulatory subunits. Two main structural families of pore-forming subunits have been identified. The first one consists of subunits with a conserved hydrophobic core containing six transmembrane domains (TMDs). These K^+ channel α subunits participate in the formation of outward rectifier voltage-gated (K_v) and Ca^{2+} -dependent
30 K^+ channels. The fourth TMD contains repeated positive charges involved in the

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voltage gating of these channels and hence in their outward rectification (Logothetis *et al.* (1992) *Neuron*, 8, 531-540; Bezanilla *et al.* (1994) *Biophys. J.* 66, 1011-1021).

The second family of pore-forming subunits have only two TMDs. They are essential subunits of inward-rectifying (IRK), G-protein-coupled (GIRK) and ATP-sensitive (K_{ATP}) K^+ channels. The inward rectification results from a voltage-dependent block by cytoplasmic Mg^{2+} and polyamines (Matsuda, H. (1991) *Annu. Rev. Physiol.*, 53, 289-298). A conserved domain, called the P domain, is present in all members of both families (Pongs, O. (1993) *J. Membr. Biol.*, 136, 1-8; Heginbotham *et al.* (1994) *Biophys. J.* 66, 1061-1067; Mackinnon, R. (1995) *Neuron*, 14, 889-892; Pascual *et al.*, (1995) *Neuron*, and 14, 1055-1063). This domain is an essential element of the aqueous K^+ -selective pore. In both groups, the assembly of four subunits is necessary to form a functional K^+ channel (Mackinnon, R. (1991) *Nature*, 350, 232-235; Yang *et al.*, (1995) *Neuron*, 15, 1441-1447).

In both six TMD and two TMD pore-forming subunit families, different subunits coded by different genes can associate to form heterotetramers with new channel properties (Isacoff *et al.*, (1990) *Nature*, 345, 530-534). A selective formation of heteropolymeric channels may allow each cell to develop the best K^+ current repertoire suited to its function. Pore-forming α subunits of Kv channels are classified into different subfamilies according to their sequence similarity (Chandy *et al.* (1993) *Trends Pharmacol. Sci.*, 14: 434). Tetramerization is believed to occur preferentially between members of each subgroup (Covarrubias *et al.* (1991) *Neuron*, 7, 763-773). The domain responsible for this selective association is localized in the N-terminal region and is conserved between members of the same subgroup. This domain is necessary for hetero- but not homo-multimeric assembly within a subfamily and prevents co-assembly between subfamilies. Recently, pore-forming subunits with two TMDs were also shown to co-assemble to form heteropolymers (Duprat *et al.* (1995) *Biochem. Biophys. Res. Commun.*, 212, 657-663). This heteropolymerization seems necessary to give functional GIRKs. IRKs are active as homopolymers but also form heteropolymers.

New structural types of K^+ channels were identified recently in both humans and yeast. These channels have two P domains in their functional subunit instead of only one (Ketchum *et al.* (1995) *Nature*, 376, 690-695; Lesage *et al.* (1996) *J. Biol. Chem.*,

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271, 4183-4187; Lesage *et al.* (1996) *EMBO J.*, 15, 1004-1011; Reid *et al.* (1996) *Receptors Channels* 4, 51-62). The human channel called TWIK-1, has four TMDs. TWIK-1 is expressed widely in human tissues and is particularly abundant in the heart and the brain. TWIK-1 currents are time independent and inwardly rectifying. These
5 properties suggest that TWIK-1 channels are involved in the control of the background K^+ membrane conductance (Lesage *et al.* (1996) *EMBO J.*, 15, 1004-1011).

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel
10 members of the TWIK (for Tandem of P domains in a Weak Inward rectifying K^+ channel)-like family of potassium channels, referred to herein as TWIK-8 nucleic acid and protein molecules. The TWIK-8 molecules of the present invention are useful as targets for developing modulating agents to regulate a variety of cellular processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules
15 encoding TWIK-8 proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of TWIK-8-encoding nucleic acids.

In one embodiment, a TWIK-8 nucleic acid molecule of the invention is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
20 97%, 98%, 99% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1 or 3 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof. In a preferred embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:1 or 3, or a complement thereof.
25 In another embodiment, the nucleic acid molecule includes SEQ ID NO:3 and nucleotides 1-83 of SEQ ID NO:1. In yet another embodiment, the nucleic acid molecule includes SEQ ID NO:3 and nucleotides 1344-1408 of SEQ ID NO:1. In another preferred embodiment, the nucleic acid molecule consists of the nucleotide sequence shown in SEQ ID NO:1 or 3. In another preferred embodiment, the nucleic
30 acid molecule includes a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250,

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1300, 1350, or 1400, nucleotides (*e.g.*, contiguous nucleotides) of the nucleotide sequence of SEQ ID NO: 1 or 3, or a complement thereof.

In another embodiment, a TWIK-8 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In a preferred embodiment, a TWIK-8 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the amino acid sequence of SEQ ID NO:2 or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of human TWIK-8. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

Another embodiment of the invention features nucleic acid molecules, preferably TWIK-8 nucleic acid molecules, which specifically detect TWIK-8 nucleic acid molecules relative to nucleic acid molecules encoding non-TWIK-8 proteins. For example, in one embodiment, such a nucleic acid molecule is at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof.

In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the nucleic acid molecule

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hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or 3 under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to a TWIK-8 nucleic acid molecule, *e.g.*, the coding strand of a
5 TWIK-8 nucleic acid molecule.

Another aspect of the invention provides a vector comprising a TWIK-8 nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. In yet another embodiment, the invention provides a host cell containing a
10 nucleic acid molecule of the invention. The invention also provides a method for producing a protein, preferably a TWIK-8 protein, by culturing in a suitable medium, a host cell, *e.g.*, a mammalian host cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant TWIK-8
15 proteins and polypeptides. In one embodiment, an isolated TWIK-8 protein includes at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an
20 outward-rectifier TOK1 potassium channel domain.

In a preferred embodiment, a TWIK-8 protein includes at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated
25 potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with
30 ATCC as Accession Number _____.

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In another preferred embodiment, a TWIK-8 protein includes one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and has a TWIK-8 activity (as described herein).

In yet another preferred embodiment, a TWIK-8 protein includes one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3.

In another embodiment, the invention features fragments of the protein having the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 15 amino acids (*e.g.*, 15 contiguous amino acids) of the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____. In another embodiment, a TWIK-8 protein has the amino acid sequence of SEQ ID NO:2.

In another embodiment, the invention features a TWIK-8 protein which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof. This invention further features a TWIK-8 protein, which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof.

The proteins of the present invention or portions thereof, *e.g.*, biologically active portions thereof, can be operatively linked to a non-TWIK-8 polypeptide (*e.g.*, heterologous amino acid sequences) to form fusion proteins. The invention further

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features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention, preferably TWIK-8 proteins. In addition, the TWIK-8 proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

5 In another aspect, the present invention provides a method for detecting the presence of a TWIK-8 nucleic acid molecule, protein, or polypeptide in a biological sample by contacting the biological sample with an agent capable of detecting a TWIK-8 nucleic acid molecule, protein, or polypeptide such that the presence of a TWIK-8 nucleic acid molecule, protein or polypeptide is detected in the biological sample.

10 In another aspect, the present invention provides a method for detecting the presence of TWIK-8 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of TWIK-8 activity such that the presence of TWIK-8 activity is detected in the biological sample.

 In another aspect, the invention provides a method for modulating TWIK-8
15 activity comprising contacting a cell capable of expressing TWIK-8 with an agent that modulates TWIK-8 activity such that TWIK-8 activity in the cell is modulated. In one embodiment, the agent inhibits TWIK-8 activity. In another embodiment, the agent stimulates TWIK-8 activity. In one embodiment, the agent is an antibody that specifically binds to a TWIK-8 protein. In another embodiment, the agent modulates
20 expression of TWIK-8 by modulating transcription of a TWIK-8 gene or translation of a TWIK-8 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a TWIK-8 mRNA or a TWIK-8 gene.

 In one embodiment, the methods of the present invention are used to treat a
25 subject having a disorder characterized by aberrant or unwanted TWIK-8 protein or nucleic acid expression or activity by administering an agent which is a TWIK-8 modulator to the subject. In one embodiment, the TWIK-8 modulator is a TWIK-8 protein. In another embodiment the TWIK-8 modulator is a TWIK-8 nucleic acid molecule. In yet another embodiment, the TWIK-8 modulator is a peptide,
30 peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant or unwanted TWIK-8 protein or nucleic acid expression is a CNS disorder, such as a cognitive or neurodegenerative disorder. In another preferred

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embodiment, the disorder characterized by aberrant or unwanted TWIK-8 protein or nucleic acid expression is a cardiovascular disorder. In another preferred embodiment, the disorder characterized by aberrant or unwanted TWIK-8 protein or nucleic acid expression is a muscular disorder. In another embodiment, the disorder characterized by aberrant or unwanted TWIK-8 activity is a cell proliferation, growth, differentiation, or migration disorder. In another embodiment, the disorder characterized by aberrant or unwanted TWIK-8 activity is a pain disorder, or a disorder characterized by misregulated pain signaling mechanisms.

The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a TWIK-8 protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a TWIK-8 protein, wherein a wild-type form of the gene encodes a protein with a TWIK-8 activity.

In another aspect the invention provides methods for identifying a compound that binds to or modulates the activity of a TWIK-8 protein, by providing an indicator composition comprising a TWIK-8 protein having TWIK-8 activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on TWIK-8 activity in the indicator composition to identify a compound that modulates the activity of a TWIK-8 protein.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 depicts the cDNA sequence and predicted amino acid sequence of human TWIK-8. The nucleotide sequence corresponds to nucleic acids 1 to 1408 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 419 of SEQ ID NO: 2. The coding region without the 3' untranslated region of the human TWIK-8 gene is shown in SEQ ID NO: 3.

Figure 2 depicts the results of an analysis of the human TWIK-8 amino acid sequence (SEQ ID NO:2) by the Signal P program (Henrik, *et al.* (1997) *Protein Engineering* 10:1-6), indicating the presence of a signal peptide at about residues 1-46 of the native molecule.

5 *Figure 3* depicts the results of a search which was performed against the MEMSAT database and which resulted in the identification of six transmembrane domains in the native human TWIK-8 protein (SEQ ID NO:2), and five transmembrane domains in the mature form of the human TWIK-8 protein.

10 *Figure 4* depicts the results of a search performed against the HMM database which identified the presence of a “seven-transmembrane receptor domain” and a “cyclic nucleotide-gated channel domain” in the amino acid sequence of human TWIK-8 (SEQ ID NO:2).

15 *Figure 5* depicts the results of a search performed against the ProDom database which identified the presence of a “TRAAK potassium channel domain”, a “potassium channel protein domain”, a “voltage-gated potassium channel domain”, an “outward-rectifier TOK1 potassium channel domain”, and a “potassium channel subunit domain” in the amino acid sequence of human TWIK-8 (SEQ ID NO:2).

Detailed Description of the Invention

20 The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as TWIK-8 nucleic acid and protein molecules, which are novel members of the TWIK (for Tandem of P domains in a Weak Inward rectifying K⁺ channel)-like family of potassium channels. These novel molecules are capable of, for example, modulating a potassium channel mediated activity in a cell, *e.g.*, a neuronal
25 cell, or a muscle cell.

As used herein, a “potassium channel” includes a protein or polypeptide which is involved in receiving, conducting, and transmitting signals in an electrically excitable or a non-electrically excitable cell, *e.g.*, a neuronal cell, or a muscle cell (*e.g.*, a cardiac muscle cell). Potassium channels are potassium ion selective, and can determine
30 membrane excitability (the ability of, for example, a neuron to respond to a stimulus and convert it into an impulse). Potassium channels can also influence the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of

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excitation. Potassium channels are typically expressed in electrically excitable cells, *e.g.*, neurons, muscle, endocrine, and egg cells, and may form heteromultimeric structures, *e.g.*, composed of pore-forming α and cytoplasmic β subunits. Potassium channels may also be found in non-excitable cells (*e.g.*, spleen cells or prostate cells),
5 where they may play a role in, *e.g.*, signal transduction. Examples of potassium channels include: (1) the voltage-gated potassium channels, (2) the ligand-gated potassium channels, *e.g.*, neurotransmitter-gated potassium channels, and (3) cyclic-nucleotide-gated potassium channels. Voltage-gated and ligand-gated potassium channels are expressed in the brain, *e.g.*, in brainstem monoaminergic and forebrain
10 cholinergic neurons, where they are involved in the release of neurotransmitters, or in the dendrites of hippocampal and neocortical pyramidal cells, where they are involved in the processes of learning and memory formation. For a detailed description of potassium channels, see Kandel E.R., *et al.*, Principles of Neural Science, second edition, (Elsevier Science Publishing Co., Inc., N.Y. (1985)), the contents of which are
15 incorporated herein by reference. As the TWIK-like proteins of the present invention may modulate potassium channel mediated activities, they may be useful for developing novel diagnostic and therapeutic agents for potassium channel associated disorders.

As used herein, a "potassium channel associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of a potassium channel
20 mediated activity. Potassium channel associated disorders can detrimentally affect conveyance of sensory impulses from the periphery to the brain and/or conductance of motor impulses from the brain to the periphery; integration of reflexes; interpretation of sensory impulses; and emotional, intellectual (*e.g.*, learning and memory), or motor processes.

25 Examples of potassium channel associated disorders include CNS disorders such as cognitive and neurodegenerative disorders, examples of which include, but are not limited to, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, senile dementia, Huntington's disease, Gilles de la Tourette's syndrome, multiple sclerosis, amyotrophic
30 lateral sclerosis, movement disorders, progressive supranuclear palsy, epilepsy, AIDS related dementia, and Jakob-Creutzfeldt disease; autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders, such as depression,

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schizophrenia, schizoaffective disorder, korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss, attention deficit disorder, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, phobias, panic disorder, as well as bipolar affective disorder, *e.g.*, severe bipolar affective (mood) disorder (BP-1), and bipolar affective neurological disorders, *e.g.*, migraine and obesity. Further CNS-related disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

Further examples of potassium channel associated disorders include cardiac-related disorders. Cardiovascular system disorders in which the TWIK-8 molecules of the invention may be directly or indirectly involved include arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, Jervell syndrome, Lange-Nielsen syndrome, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, and arrhythmia. TWIK-8-mediated or related disorders also include disorders of the musculoskeletal system such as paralysis and muscle weakness, *e.g.*, ataxia, myotonia, and myokymia.

Other examples of potassium channel-associated disorders include pain disorders. Pain disorders include those disorders that affect pain signaling mechanisms. As used herein, the term "pain signaling mechanisms" includes the cellular mechanisms involved in the development and regulation of pain, *e.g.*, pain elicited by noxious chemical, mechanical, or thermal stimuli, in a subject, *e.g.*, a mammal such as a human. In mammals, the initial detection of noxious chemical, mechanical, or thermal stimuli, a process referred to as "nociception", occurs predominantly at the peripheral terminals of specialized, small diameter sensory neurons. These sensory neurons transmit the information to the central nervous system, evoking a perception of pain or discomfort and initiating appropriate protective reflexes. The TWIK-8 molecules of the present

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invention may be present on these sensory neurons and, thus, may be involved in detecting these noxious chemical, mechanical, or thermal stimuli and transducing this information into membrane depolarization events. Thus, the TWIK-8 molecules by participating in pain signaling mechanisms, may modulate pain elicitation and act as targets for developing novel diagnostic targets and therapeutic agents to control pain. Examples of pain disorders include headache posttherapeutic neuralgia, diabetic neuropathy, postmastectomy pain syndrome, stump pain, reflex sympathetic dystrophy, trigeminal neuralgia, neuropathic pain, orofacial neuropathic pain, osteoarthritis, arthritis, *e.g.*, rheumatoid arthritis, fibromyalgia syndrome, tension myalgia, Guillian-Barre syndrome, Meralgia paraesthetica, burning mouth syndrome, fibrocitis, myofascial pain syndrome, idiopathic pain disorder, temporomandibular joint syndrome, atypical odontalgia, loin pain, haematuria syndrome, non-cardiac chest pain, back pain, chronic nonspecific pain, pain associated with surgery, psychogenic pain, tooth pain, musculoskeletal pain disorder, chronic pelvic pain, nonorganic chronic headache, tension-type headache, cluster headache, migraine, complex regional pain syndrome, vaginismus, nerve trunk pain, somatoform pain disorder, cyclical mastalgia, chronic fatigue syndrome, multiple somatization syndrome, chronic pain disorder, cancer pain, somatization disorder, Syndrome X, facial pain, idiopathic pain disorder, posttraumatic rheumatic pain modulation disorder (fibrositis syndrome), hyperalgesia, and Tangier disease.

Potassium channel disorders also include cellular proliferation, growth, differentiation, or migration disorders. Cellular proliferation, growth, differentiation, or migration disorders include those disorders that affect cell proliferation, growth, differentiation, or migration processes. As used herein, a "cellular proliferation, growth, differentiation, or migration process" is a process by which a cell increases in number, size or content, by which a cell develops a specialized set of characteristics which differ from that of other cells, or by which a cell moves closer to or further from a particular location or stimulus. The TWIK-8 molecules of the present invention are involved in signal transduction mechanisms, which are known to be involved in cellular growth, differentiation, and migration processes. Thus, the TWIK-8 molecules may modulate cellular growth, differentiation, or migration, and may play a role in disorders characterized by aberrantly regulated growth, differentiation, or migration. Such

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disorders include cancer, *e.g.*, carcinoma, sarcoma, or leukemia; tumor angiogenesis and metastasis; skeletal dysplasia; neuronal deficiencies resulting from impaired neural induction and patterning; and hematopoietic and/or myeloproliferative disorders.

TWIK-8-associated or related disorders also include disorders of tissues in which
5 TWIK-8 protein is expressed, *e.g.*, cortex, hypothalamus and dorsal root ganglia.

As used herein, a "potassium channel mediated activity" includes an activity which involves a potassium channel, *e.g.*, a potassium channel in a neuronal cell, or a muscle cell (*e.g.*, a cardiac muscle cell), associated with receiving, conducting, and transmitting signals in, for example, the nervous system. Potassium channel mediated
10 activities include release of neurotransmitters, *e.g.*, dopamine or norepinephrine, from cells, *e.g.*, neuronal cells; modulation of resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation; and modulation of processes such as integration of sub-threshold synaptic responses, the conductance of back-propagating action potentials in, for example, neuronal cells or muscle cells,
15 participation in signal transduction pathways, and participation in nociception.

The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-
20 naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin, *e.g.*, monkey proteins. Members of a family may also have common functional characteristics.

For example, the family of TWIK-8 proteins comprises at least one
25 "transmembrane domain" and preferably six transmembrane domains. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid
30 residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an alpha-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a

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transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta W.N. *et al.* (1996) *Annual Rev. Neurosci.* 19: 235-263, the contents of which are incorporated herein by reference. Amino acid residues 32-50, 116-137, 144-165, 195-219, 226-242, 5 and 260-283 of the native TWIK-8 protein, and amino acid residues 70-91, 98-119, 149-173, 180-196 and 214-237 of the mature TWIK-8 protein are predicted to comprise transmembrane domains (see Figure 3). Accordingly, TWIK-8 proteins having at least 50-60% homology, preferably about 60-70%, more preferably about 70-80%, or about 80-90% homology with a transmembrane domain of human TWIK-8 are within the 10 scope of the invention.

In another embodiment, a TWIK-8 molecule of the present invention is identified based on the presence of a Pore loop (P-loop). As used herein, the term "Pore loop" or "P-loop" includes an amino acid sequence of about 15-45 amino acid residues in length, preferably about 15-35 amino acid residues in length, and most preferably 15 about 15-25 amino acid residues in length, which is involved in lining the potassium channel pore. A P-loop is typically found between transmembrane domains of potassium channels and is believed to be a major determinant of ion selectivity in potassium channels. Preferably, P-loops contain a G-[HYDROPHOBIC AMINO ACID]-G sequence, *e.g.*, a GYG, GLG, or GFG sequence. P-loops are described in, for 20 example, Warmke *et al.* (1991) *Science* 252:1560-1562; Zagotta W.N. *et al.*, (1996) *Annual Rev. Neurosci.* 19:235-63 (Pongs, O. (1993) *J. Membr. Biol.*, 136, 1-8; Heginbotham *et al.* (1994) *Biophys. J.* 66,1061-1067; Mackinnon, R. (1995) *Neuron*, and 14, 889-892; Pascual *et al.*, (1995) *Neuron*, 14, 1055-1063), the contents of which are incorporated herein by reference. Amino acid residues 243-259 of the native human 25 TWIK-8 protein, and residues 197-213 of the predicted mature human TWIK-8 protein comprise a P-loop.

In a preferred embodiment, the TWIK-8 molecules of the invention include at least one and, preferably, six transmembrane domains and at least one P-loop domain.

In another embodiment, a TWIK-8 molecule of the present invention is 30 identified based on the presence of a "seven-transmembrane receptor domain" in the protein or corresponding nucleic acid molecule. Seven-transmembrane receptor domains are described, for example, in Hamann *et al.* (1996) *Genomics* 32: 144-147. As

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used herein, the term “seven-transmembrane receptor domain” includes a protein domain having an amino acid sequence of about 150-320 amino acid residues.

Preferably, a seven-transmembrane receptor domain includes at least about 200-250, or more preferably about 220 amino acid residues. To identify the presence of a seven-

5 transmembrane receptor domain in a TWIK-8 protein, and to make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of known protein domains (*e.g.*, the HMM database). The seven-transmembrane receptor domain (HMM) has been assigned the PFAM Accession PF00002 (<http://genome.wustl.edu/Pfam/html>). A search was performed against the
10 HMM database resulting in the identification of a seven-transmembrane receptor domain in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2) at about residues 25-244 of SEQ ID NO: 2. The results of the search are set forth in Figure 4.

In another embodiment, a TWIK-8 molecule of the present invention is identified based on the presence of a “cyclic nucleotide-gated channel domain” in the
15 protein or corresponding nucleic acid molecule. Cyclic nucleotide-gated channel domains are described, for example, in Yau (1994) *Proc. Natl. Acad. Sci. USA* 91: 3481-3483. As used herein, the term “cyclic nucleotide-gated channel domain” includes a protein domain having an amino acid sequence of about 100-225 amino acid residues. Preferably, a cyclic nucleotide-gated channel domain includes at least about 150-200, or
20 more preferably about 178 amino acid residues. To identify the presence of a cyclic nucleotide-gated channel domain in a TWIK-8 protein, and to make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of known protein domains (*e.g.*, the HMM database). The cyclic nucleotide-gated channel domain (HMM) has been assigned the PFAM Accession
25 PF00914 (<http://genome.wustl.edu/Pfam/html>). A search was performed against the HMM database resulting in the identification of a cyclic nucleotide-gated channel domain in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2) at about residues 27-204 of SEQ ID NO: 2. The results of the search are set forth in Figure 4.

In another embodiment, a TWIK-8 molecule of the present invention is
30 identified based on the presence of a “TRAAK potassium channel domain” in the protein or corresponding nucleic acid molecule. As used herein, the term “TRAAK potassium channel domain” includes a protein domain having an amino acid sequence of

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about 20-150 amino acid residues and having a bit score for the alignment of the sequence to the TRAAK potassium channel domain of at least 115-175. Preferably, a TRAAK potassium channel domain includes at least about 23-100, or more preferably about 25, 55, or 95 amino acid residues, and has a bit score for the alignment of the sequence to the TRAAK potassium channel domain of at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 or higher. The TRAAK potassium channel domain has been assigned ProDom entries 73512, 98483, and 105542. To identify the presence of a TRAAK potassium channel domain in a TWIK-8 protein, and to make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of known protein domains (*e.g.*, the ProDom database) using the default parameters (available at <http://www.toulouse.inra.fr/prodom.html>). A search was performed against the ProDom database resulting in the identification of a TRAAK potassium channel domains in the amino acid sequence of human TWIK-8 (SEQ ID NO:2) at about residues 50-104, 175-199, and 288-382 of SEQ ID NO: 2. The results of the search are set forth in Figures 5A, 5B, and 5D.

In another embodiment, a TWIK-8 molecule of the present invention is identified based on the presence of a "potassium channel protein domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "potassium channel protein domain" includes a protein domain having an amino acid sequence of about 20-100 amino acid residues and having a bit score for the alignment of the sequence to the potassium channel protein domain of at least 101. Preferably, a potassium channel protein domain includes at least about 40-75, or more preferably about 55 amino acid residues, and has a bit score for the alignment of the sequence to the potassium channel protein domain of at least 20, 30, 40, 50, 60, 70, 80, 90, 100, or higher. The potassium channel protein domain has been assigned ProDom entry 129403. To identify the presence of a potassium channel protein domain in a TWIK-8 protein, and to make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of known protein domains (*e.g.*, the ProDom database) using the default parameters (available at <http://www.toulouse.inra.fr/prodom.html>). A search was performed against the ProDom database resulting in the identification of a potassium channel protein domain in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2) at

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about residues 99-153 of SEQ ID NO: 2. The results of the search are set forth in Figure 5F.

In another embodiment, a TWIK-8 molecule of the present invention is identified based on the presence of a "voltage-gated potassium channel domain" in the protein or
5 corresponding nucleic acid molecule. As used herein, the term "voltage-gated potassium channel domain" includes a protein domain having an amino acid sequence of about 20-100 amino acid residues and having a bit score for the alignment of the sequence to the potassium channel protein domain of at least 115. Preferably, a voltage-gated potassium channel domain includes at least about 40-75, or more preferably about 55 amino acid
10 residues, and has a bit score for the alignment of the sequence to the voltage-gated potassium channel domain of at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 or higher. The voltage-gated potassium channel domain has been assigned ProDom entry 36. To identify the presence of a voltage-gated potassium channel domain in a TWIK-8 protein, and to make the determination that a protein of interest has a particular profile, the amino acid
15 sequence of the protein is searched against a database of known protein domains (*e.g.*, the ProDom database) using the default parameters (available at <http://www.toulouse.inra.fr/prodom.html>). A search was performed against the ProDom database resulting in the identification of a voltage-gated potassium channel domain in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2) at about residues 102-168 of SEQ
20 ID NO: 2. The results of the search are set forth in Figure 5E.

In another embodiment, a TWIK-8 molecule of the present invention is identified based on the presence of an "outward-rectifier TOK1 potassium channel domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "outward-rectifier TOK1 potassium channel domain" includes a protein domain having an amino
25 acid sequence of about 25-100 amino acid residues and having a bit score for the alignment of the sequence to the outward-rectifier TOK1 potassium channel domain of at least 70. Preferably, an outward-rectifier TOK1 potassium channel domain includes at least about 40-75, or more preferably about 56 amino acid residues, and has a bit score for the alignment of the sequence to the outward-rectifier TOK1 potassium channel domain of at
30 least 20, 30, 40, 50, 60, or higher. The outward-rectifier TOK1 potassium channel domain has been assigned ProDom entry 32818. To identify the presence of an outward-rectifier TOK1 potassium channel domain in a TWIK-8 protein, and to make the determination that

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a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of known protein domains (*e.g.*, the ProDom database) using the default parameters (available at <http://www.toulouse.inra.fr/prodom.html>). A search was performed against the ProDom database resulting in the identification of an outward-rectifier TOK1 potassium channel domain in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2) at about residues 215-270 of SEQ ID NO: 2. The results of the search are set forth in Figure 5G.

In another embodiment, a TWIK-8 molecule of the present invention is identified based on the presence of a "potassium channel subunit domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "potassium channel subunit domain" includes a protein domain having an amino acid sequence of about 25-125 amino acid residues and having a bit score for the alignment of the sequence to the potassium channel subunit domain of at least 156. Preferably, a potassium channel subunit domain includes at least about 40-100, or more preferably about 72 amino acid residues, and has a bit score for the alignment of the sequence to the potassium channel subunit domain of at least 20, 30, 40, 50, 60, or higher. The potassium channel subunit domain has been assigned ProDom entry 1641. To identify the presence of a potassium channel subunit domain in a TWIK-8 protein, and to make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of known protein domains (*e.g.*, the ProDom database) using the default parameters (available at <http://www.toulouse.inra.fr/prodom.html>). A search was performed against the ProDom database resulting in the identification of a potassium channel subunit domain in the amino acid sequence of human TWIK-8 (SEQ ID NO: 2) at about residues 216-287 of SEQ ID NO: 2. The results of the search are set forth in Figure 5C.

Isolated proteins of the present invention, preferably TWIK-8 proteins, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:1 or 3. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional

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activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% homology, preferably 60% homology, more preferably 70%-80%, and even more preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95% homology and share a common functional activity are defined herein as sufficiently identical.

As used interchangeably herein, an "TWIK-8 activity", "biological activity of TWIK-8" or "functional activity of TWIK-8", refers to an activity exerted by a TWIK-8 protein, polypeptide or nucleic acid molecule on a TWIK-8 responsive cell or tissue, or on a TWIK-8 protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a TWIK-8 activity is a direct activity, such as an association with a TWIK-8-target molecule. As used herein, a "target molecule" or "binding partner" is a molecule with which a TWIK-8 protein binds or interacts in nature, such that TWIK-8-mediated function is achieved. A TWIK-8 target molecule can be a non-TWIK-8 molecule or a TWIK-8 protein or polypeptide of the present invention. In an exemplary embodiment, a TWIK-8 target molecule is a TWIK-8 ligand, *e.g.*, a potassium channel pore-forming subunit or a potassium channel ligand. Alternatively, a TWIK-8 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the TWIK-8 protein with a TWIK-8 ligand. The biological activities of TWIK-8 are described herein. For example, the TWIK-8 proteins of the present invention can have one or more of the following activities: (1) interacting with a non-TWIK protein molecule; (2) activating a TWIK-dependent signal transduction pathway; (3) modulating the release of neurotransmitters; (4) modulating membrane excitability; (5) influencing the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation, (6) modulating processes which underlie learning and memory, such as integration of sub-threshold synaptic responses and the conductance of back-propagating action potentials, and (7) mediating nociception.

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Accordingly, another embodiment of the invention features isolated TWIK-8 proteins and polypeptides having a TWIK-8 activity. Preferred proteins are TWIK-8 proteins having at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and, preferably, a TWIK-8 activity.

Additional preferred proteins have at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3.

The nucleotide sequence of the isolated human TWIK-8 cDNA and the predicted amino acid sequence of the human TWIK-8 polypeptide are shown in Figure 1 and in SEQ ID NOs:1 and 2, respectively. A plasmid containing the nucleotide sequence encoding human TWIK-8 was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The human TWIK-8 gene, which is approximately 1408 nucleotides in length, encodes a protein having a molecular weight of approximately 46.1 kD and which is approximately 419 amino acid residues in length.

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Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

5 One aspect of the invention pertains to isolated nucleic acid molecules that encode TWIK-8 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify TWIK-8-encoding nucleic acid molecules (*e.g.*, TWIK-8 mRNA) and fragments for use as PCR primers for the amplification or mutation of TWIK-8 nucleic acid molecules. As used herein, the
10 term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

 The term "isolated nucleic acid molecule" includes nucleic acid molecules which
15 are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and
20 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated TWIK-8 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid
25 molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

 A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the
30 DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence

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of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, as a hybridization probe, TWIK-8 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989*).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to TWIK-8 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the human TWIK-8 cDNA. This cDNA comprises sequences encoding the human TWIK-8 protein (*i.e.*, "the coding region", from nucleotides 84-1343), as well as 5' untranslated sequences (nucleotides 1-83) and 3' untranslated sequences (nucleotides 1344-1408). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 84-1343, corresponding to SEQ ID NO:3).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the

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DNA insert of the plasmid deposited with ATCC as Accession Number _____, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, such that it can hybridize to the nucleotide sequence
5 shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%,
10 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1 or 3, or the entire length of the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences.

15 Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a TWIK-8 protein, *e.g.*, a biologically active portion of a TWIK-8 protein.
20 The nucleotide sequence determined from the cloning of the TWIK-8 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other TWIK-8 family members, as well as TWIK-8 homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes
25 under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, 75, 80, 85, 90, 95, 100, 150, or 200 or more consecutive nucleotides of a sense sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, of an anti-sense sequence of SEQ ID NO:1 or 3, or the
30 nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited

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with ATCC as Accession Number _____. In one embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500 or more nucleotides
5 in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

Probes based on the TWIK-8 nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In
10 preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a TWIK-8 protein, such as by measuring a level of a TWIK-8-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting TWIK-8
15 mRNA levels or determining whether a genomic TWIK-8 gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a TWIK-8 protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with
20 ATCC as Accession Number _____, which encodes a polypeptide having a TWIK-8 biological activity (the biological activities of the TWIK-8 proteins are described herein), expressing the encoded portion of the TWIK-8 protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the TWIK-8 protein.

25 The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, due to degeneracy of the genetic code and thus encode the same TWIK-8 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide
30 sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In another embodiment, an isolated nucleic acid molecule of the invention has a

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nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2.

In addition to the TWIK-8 nucleotide sequences shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as
5 Accession Number _____, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the TWIK-8 proteins may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the TWIK-8 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant
10 gene" refer to nucleic acid molecules which include an open reading frame encoding a TWIK-8 protein, preferably a mammalian TWIK-8 protein, and can further include non-coding regulatory sequences, and introns.

Allelic variants of human TWIK-8 include both functional and non-functional TWIK-8 proteins. Functional allelic variants are naturally occurring amino acid
15 sequence variants of the human TWIK-8 protein that maintain the ability to bind a TWIK-8 ligand or substrate and/or modulate pain signaling mechanisms, membrane excitability or neurotransmitter release. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the
20 protein.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human TWIK-8 protein that do not have the ability to either bind a TWIK-8 ligand and/or modulate any of the TWIK-8 activities described herein. Non-functional allelic variants will typically contain a non-conservative substitution, a
25 deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human orthologues of the human TWIK-8 protein. Orthologues of the human TWIK-8 protein are proteins that are isolated from non-human organisms and possess the same TWIK-8 ligand binding
30 and/or modulation of membrane excitability activities of the human TWIK-8 protein. Orthologues of the human TWIK-8 protein can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2.

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Moreover, nucleic acid molecules encoding other TWIK-8 family members and, thus, which have a nucleotide sequence which differs from the TWIK-8 sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope
5 of the invention. For example, another TWIK-8 cDNA can be identified based on the nucleotide sequence of human TWIK-8. Moreover, nucleic acid molecules encoding TWIK-8 proteins from different species, and which, thus, have a nucleotide sequence which differs from the TWIK-8 sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number
10 _____ are intended to be within the scope of the invention. For example, a mouse TWIK-8 cDNA can be identified based on the nucleotide sequence of a human TWIK-8.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the TWIK-8 cDNAs of the invention can be isolated based on their homology to the TWIK-8 nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion
15 thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural allelic variants and homologues of the TWIK-8 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the TWIK-8 gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the
20 invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In other embodiment, the nucleic acid is at least 950-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500 or
25 more nucleotides in length.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more
30 preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*,

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Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(^{\circ}\text{C}) = 2(\# \text{ of A} + \text{T bases}) + 4(\# \text{ of G} + \text{C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G} + \text{C}) - (600/\text{N})$, where N is the number of bases in the hybrid, and $[\text{Na}^+]$ is the concentration of sodium ions in the hybridization buffer ($[\text{Na}^+]$ for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH₂PO₄, 7% SDS at about 65°C, followed by

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one or more washes at 0.02M NaH₂PO₄, 1% SDS at 65°C, see *e.g.*, Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 or 3 and corresponds to a
5 naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the TWIK-8 sequences that may exist in the population, the skilled artisan will further appreciate that changes can be
10 introduced by mutation into the nucleotide sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby leading to changes in the amino acid sequence of the encoded TWIK-8 proteins, without altering the functional ability of the TWIK-8 proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-
15 essential" amino acid residues can be made in the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of TWIK-8 (*e.g.*, the sequence of SEQ ID NO:2) without altering the biological activity, whereas an "essential" amino acid residue is
20 required for biological activity. For example, amino acid residues that are conserved among the TWIK-8 proteins of the present invention, *e.g.*, those present in a transmembrane domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the TWIK-8 proteins of the present invention and other members of the TWIK family are not likely to
25 be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding TWIK-8 proteins that contain changes in amino acid residues that are not essential for activity. Such TWIK-8 proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity. In one embodiment, the isolated nucleic acid
30 molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%,

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85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2.

An isolated nucleic acid molecule encoding a TWIK-8 protein identical to the protein of SEQ ID NO:2, can be created by introducing one or more nucleotide
5 substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited
10 with ATCC as Accession Number _____ by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues
15 having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-
20 branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a TWIK-8 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a TWIK-8 coding sequence, such as by
25 saturation mutagenesis, and the resultant mutants can be screened for TWIK-8 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

30 In a preferred embodiment, a mutant TWIK-8 protein can be assayed for the ability to (1) interact with a non-TWIK protein molecule; (2) activate a TWIK-dependent signal transduction pathway; (3) modulate the release of neurotransmitters;

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(4) modulate membrane excitability; (5) influence the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation, (6) modulate processes which underlie learning and memory, such as integration of sub-threshold synaptic responses and the conductance of back-propagating action potentials, and (7) mediate nociception.

In addition to the nucleic acid molecules encoding TWIK-8 proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire TWIK-8 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding TWIK-8. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of human TWIK-8 corresponds to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding TWIK-8. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding TWIK-8 disclosed herein (*e.g.*, SEQ ID NO:3), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of TWIK-8 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of TWIK-8 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of TWIK-8 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be

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chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

- 5 Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-
10 methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-
15 thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from
20 the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

- The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a TWIK-8 protein to thereby inhibit expression of the
25 protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct
30 injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to

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receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave TWIK-8 mRNA transcripts to thereby inhibit translation of TWIK-8 mRNA. A ribozyme having specificity for a TWIK-8-encoding nucleic acid can be designed based upon the nucleotide sequence of a TWIK-8 cDNA disclosed herein (*i.e.*, SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a TWIK-8-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, TWIK-8 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, TWIK-8 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the TWIK-8 (*e.g.*, the TWIK-8 promoter and/or enhancers; *e.g.*, nucleotides 1-83 of SEQ ID NO:1) to form triple

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helical structures that prevent transcription of the TWIK-8 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the TWIK-8 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of TWIK-8 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of TWIK-8 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of TWIK-8 can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of TWIK-8 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA

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portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

Alternatively, the expression characteristics of an endogenous TWIK-8 gene within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous TWIK-8 gene. For example, an endogenous TWIK-8 gene which is normally "transcriptionally silent", *i.e.*, a TWIK-8 gene which is normally not expressed, or is expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a

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transcriptionally silent, endogenous TWIK-8 gene may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous TWIK-8 gene, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described, *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

II. Isolated TWIK-8 Proteins and Anti-TWIK-8 Antibodies

One aspect of the invention pertains to isolated TWIK-8 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-TWIK-8 antibodies. In one embodiment, native TWIK-8 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, TWIK-8 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a TWIK-8 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the TWIK-8 protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of TWIK-8 protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of TWIK-8 protein having less than about 30% (by dry weight) of non-TWIK-8 protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-TWIK-8 protein, still more preferably less than about 10% of non-TWIK-8 protein, and most preferably less than about 5% non-TWIK-8 protein. When the TWIK-8 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably

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less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of TWIK-8 protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of TWIK-8 protein having less than about 30% (by dry weight) of chemical precursors or non-TWIK-8 chemicals, more preferably less than about 20% chemical precursors or non-TWIK-8 chemicals, still more preferably less than about 10% chemical precursors or non-TWIK-8 chemicals, and most preferably less than about 5% chemical precursors or non-TWIK-8 chemicals.

As used herein, a "biologically active portion" of a TWIK-8 protein includes a fragment of a TWIK-8 protein which participates in an interaction between a TWIK-8 molecule and a non-TWIK-8 molecule. Biologically active portions of a TWIK-8 protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the TWIK-8 protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length TWIK-8 proteins, and exhibit at least one activity of a TWIK-8 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the TWIK-8 protein, *e.g.*, modulating membrane excitability. A biologically active portion of a TWIK-8 protein can be a polypeptide which is, for example, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200 or more amino acids in length. Biologically active portions of a TWIK-8 protein can be used as targets for developing agents which modulate a TWIK-8 mediated activity, *e.g.*, modulation of membrane excitability.

In one embodiment, a biologically active portion of a TWIK-8 protein comprises at least one or more of the following domains: a transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain. It is to be understood that a preferred biologically active portion of a TWIK-8 protein of the present invention may contain at least one transmembrane domain and one or more of the following domains: a

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transmembrane domain, a pore loop domain, a seven-transmembrane receptor domain, a cyclic nucleotide-gated channel domain, a TRAAK potassium channel domain, a potassium channel protein domain, a voltage-gated potassium channel domain, a potassium channel subunit domain, and an outward-rectifier TOK1 potassium channel domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native TWIK-8 protein.

In a preferred embodiment, the TWIK-8 protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the TWIK-8 protein is substantially identical to SEQ ID NO:2, and retains the functional activity of the protein of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the TWIK-8 protein is a protein which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the TWIK-8 amino acid sequence of SEQ ID NO:2 having 419 amino acid residues, at least 120, preferably at least 160, more preferably at least 201, even more preferably at least 241, and even more preferably at least 281 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a

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function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to TWIK-8 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 100, wordlength = 3 to obtain amino acid sequences homologous to TWIK-8 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

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The invention also provides TWIK-8 chimeric or fusion proteins. As used herein, a TWIK-8 "chimeric protein" or "fusion protein" comprises a TWIK-8 polypeptide operatively linked to a non-TWIK-8 polypeptide. An "TWIK-8 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to TWIK-8, whereas a "non-TWIK-8 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the TWIK-8 protein, *e.g.*, a protein which is different from the TWIK-8 protein and which is derived from the same or a different organism. Within a TWIK-8 fusion protein the TWIK-8 polypeptide can correspond to all or a portion of a TWIK-8 protein. In a preferred embodiment, a TWIK-8 fusion protein comprises at least one biologically active portion of a TWIK-8 protein. In another preferred embodiment, a TWIK-8 fusion protein comprises at least two biologically active portions of a TWIK-8 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the TWIK-8 polypeptide and the non-TWIK-8 polypeptide are fused in-frame to each other. The non-TWIK-8 polypeptide can be fused to the N-terminus or C-terminus of the TWIK-8 polypeptide.

For example, in one embodiment, the fusion protein is a GST-TWIK-8 fusion protein in which the TWIK-8 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant TWIK-8.

In another embodiment, the fusion protein is a TWIK-8 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of TWIK-8 can be increased through use of a heterologous signal sequence.

The TWIK-8 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The TWIK-8 fusion proteins can be used to affect the bioavailability of a TWIK-8 substrate. Use of TWIK-8 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a TWIK-8 protein; (ii) mis-regulation of the TWIK-8 gene; and (iii) aberrant post-translational modification of a TWIK-8 protein.

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Moreover, the TWIK-8-fusion proteins of the invention can be used as immunogens to produce anti-TWIK-8 antibodies in a subject, to purify TWIK-8 ligands and in screening assays to identify molecules which inhibit the interaction of TWIK-8 with a TWIK-8 substrate.

5 Preferably, a TWIK-8 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini,
10 filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene
15 fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A TWIK-8-encoding nucleic acid can be cloned into such an expression vector such that the fusion
20 moiety is linked in-frame to the TWIK-8 protein.

 The present invention also pertains to variants of the TWIK-8 proteins which function as either TWIK-8 agonists (mimetics) or as TWIK-8 antagonists. Variants of the TWIK-8 proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a TWIK-8 protein. An agonist of the TWIK-8 proteins can retain
25 substantially the same, or a subset, of the biological activities of the naturally occurring form of a TWIK-8 protein. An antagonist of a TWIK-8 protein can inhibit one or more of the activities of the naturally occurring form of the TWIK-8 protein by, for example, competitively modulating a TWIK-8-mediated activity of a TWIK-8 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function.
30 In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects

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in a subject relative to treatment with the naturally occurring form of the TWIK-8 protein.

In one embodiment, variants of a TWIK-8 protein which function as either TWIK-8 agonists (mimetics) or as TWIK-8 antagonists can be identified by screening
5 combinatorial libraries of mutants, *e.g.*, truncation mutants, of a TWIK-8 protein for TWIK-8 protein agonist or antagonist activity. In one embodiment, a variegated library of TWIK-8 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of TWIK-8 variants can be produced by, for example, enzymatically ligating a mixture of synthetic
10 oligonucleotides into gene sequences such that a degenerate set of potential TWIK-8 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of TWIK-8 sequences therein. There are a variety of methods which can be used to produce libraries of potential TWIK-8 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a
15 degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential TWIK-8 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science*
20 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a TWIK-8 protein coding sequence can be used to generate a variegated population of TWIK-8 fragments for screening and subsequent selection of variants of a TWIK-8 protein. In one embodiment, a library of
25 coding sequence fragments can be generated by treating a double stranded PCR fragment of a TWIK-8 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed
30 duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which

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encodes N-terminal, C-terminal and internal fragments of various sizes of the TWIK-8 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA
5 libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TWIK-8 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the
10 resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify TWIK-8 variants (Arkin and Yourvan
15 (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3): 327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated TWIK-8 library. For example, a library of expression vectors can be transfected into a cell line, *e.g.*, a neuronal cell line, which ordinarily responds to a TWIK-8 ligand in a
20 particular TWIK-8 ligand-dependent manner. The transfected cells are then contacted with a TWIK-8 ligand and the effect of expression of the mutant on, *e.g.*, membrane excitability of TWIK-8 can be detected. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the TWIK-8 ligand, and the individual clones further characterized.

25 An isolated TWIK-8 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind TWIK-8 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length TWIK-8 protein can be used or, alternatively, the invention provides antigenic peptide fragments of TWIK-8 for use as immunogens. The antigenic peptide of TWIK-8 comprises at least 8 amino acid
30 residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of TWIK-8 such that an antibody raised against the peptide forms a specific immune complex with TWIK-8. Preferably, the antigenic peptide comprises at least 10

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amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of TWIK-8 that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A TWIK-8 immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed TWIK-8 protein or a chemically synthesized TWIK-8 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic TWIK-8 preparation induces a polyclonal anti-TWIK-8 antibody response.

Accordingly, another aspect of the invention pertains to anti-TWIK-8 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as TWIK-8. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind TWIK-8. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of TWIK-8. A monoclonal antibody composition thus typically displays a single binding affinity for a particular TWIK-8 protein with which it immunoreacts.

Polyclonal anti-TWIK-8 antibodies can be prepared as described above by immunizing a suitable subject with a TWIK-8 immunogen. The anti-TWIK-8 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized TWIK-8. If desired, the antibody molecules directed against TWIK-8 can be isolated from the

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mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-TWIK-8 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a TWIK-8 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds TWIK-8.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-TWIK-8 monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques,

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e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused
5 and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind TWIK-8, *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a
10 monoclonal anti-TWIK-8 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with TWIK-8 to thereby isolate immunoglobulin library members that bind TWIK-8. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-
15 9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.*
20 PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-
25 1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc.*
30 *Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.

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Additionally, recombinant anti-TWIK-8 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-TWIK-8 antibody (*e.g.*, monoclonal antibody) can be used to isolate TWIK-8 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-TWIK-8 antibody can facilitate the purification of natural TWIK-8 from cells and of recombinantly produced TWIK-8 expressed in host cells. Moreover, an anti-TWIK-8 antibody can be used to detect TWIK-8 protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the TWIK-8 protein. Anti-TWIK-8 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable

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fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable

5 radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a TWIK-8 protein (or a portion thereof). As
10 used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of
15 autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to
20 which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors,
25 such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory
30 sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of

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interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, TWIK-8 proteins, mutant forms of TWIK-8 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of TWIK-8 proteins in prokaryotic or eukaryotic cells. For example, TWIK-8 proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion

moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in TWIK-8 activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for TWIK-8 proteins, for example. In a preferred embodiment, a TWIK-8 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118).

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Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the TWIK-8 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1
5 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, TWIK-8 proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins
10 in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian
15 expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both
20 prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed.*, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is
25 capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters

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(e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-
5 regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense
10 orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to TWIK-8 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of
15 cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be
20 determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a TWIK-8
25 nucleic acid molecule of the invention is introduced, e.g., a TWIK-8 nucleic acid molecule within a recombinant expression vector or a TWIK-8 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular
30 subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or

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environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a TWIK-8 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or
5 mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized
10 techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor*
15 *Laboratory Press, Cold Spring Harbor, NY, 1989*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is
20 generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a TWIK-8 protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic
25 acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a TWIK-8 protein. Accordingly, the invention further provides methods for producing a TWIK-8 protein using the host cells
30 of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a TWIK-8 protein has been introduced) in a suitable medium such that a TWIK-8 protein is produced. In

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another embodiment, the method further comprises isolating a TWIK-8 protein from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized
5 oocyte or an embryonic stem cell into which TWIK-8-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous TWIK-8 sequences have been introduced into their genome or homologous recombinant animals in which endogenous TWIK-8 sequences have been altered. Such animals are useful for studying the function and/or activity of a TWIK-8
10 and for identifying and/or evaluating modulators of TWIK-8 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA
15 which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous TWIK-8 gene has been
20 altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a TWIK-8-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by
25 microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The TWIK-8 cDNA sequence of SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human TWIK-8 gene, such as a mouse or rat TWIK-8 gene, can be used as a transgene. Alternatively, a TWIK-8 gene homologue, such as
30 another TWIK-8 family member, can be isolated based on hybridization to the TWIK-8 cDNA sequences of SEQ ID NO:1 or 3, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____ (described further in subsection I above) and used

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as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a TWIK-8 transgene to direct expression of a TWIK-8 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a TWIK-8 transgene in its genome and/or expression of TWIK-8 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a TWIK-8 protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a TWIK-8 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the TWIK-8 gene. The TWIK-8 gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:3), but more preferably, is a non-human homologue of a human TWIK-8 gene (*e.g.*, a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1). For example, a mouse TWIK-8 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous TWIK-8 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous TWIK-8 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous TWIK-8 gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous TWIK-8 protein). In the homologous recombination nucleic acid molecule, the altered portion of the TWIK-8 gene is flanked at its 5' and 3'

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ends by additional nucleic acid sequence of the TWIK-8 gene to allow for homologous recombination to occur between the exogenous TWIK-8 gene carried by the homologous recombination nucleic acid molecule and an endogenous TWIK-8 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking TWIK-8 nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced TWIK-8 gene has homologously recombined with the endogenous TWIK-8 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then be injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a

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selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

5 Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be
10 fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

15

IV. Pharmaceutical Compositions

The TWIK-8 nucleic acid molecules, fragments of TWIK-8 proteins, and anti-TWIK-8 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such
20 compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and
25 agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible
30 with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions

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used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

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Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of a TWIK-8 protein or an anti-TWIK-8 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions
5 are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a
10 previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared
15 using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as
20 microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

25 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be
30 permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the

use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with
5 conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems.
10 Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected
15 cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form
20 as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound
25 and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose
30 therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. While

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compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in
5 formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially
10 from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high
15 performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to
20 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can
25 include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be
30 appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in

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dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram.

It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the

activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

5 Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy
10 anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil,
15 melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and
20 vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin
25 such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"),
30 granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

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Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and

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prophylactic). As described herein, a TWIK-8 protein of the invention has one or more of the following activities: (1) interacting with a non-TWIK protein molecule; (2) activating a TWIK-dependent signal transduction pathway; (3) modulating the release of neurotransmitters; (4) modulating membrane excitability; (5) influencing the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation, (6) modulating processes which underlie learning and memory, such as integration of sub-threshold synaptic responses and the conductance of back-propagating action potentials, and (7) mediating nociception.

The isolated nucleic acid molecules of the invention can be used, for example, to express TWIK-8 protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect TWIK-8 mRNA (*e.g.*, in a biological sample) or a genetic alteration in a TWIK-8 gene, and to modulate TWIK-8 activity, as described further below. The TWIK-8 proteins can be used to treat disorders characterized by insufficient or excessive production of a TWIK-8 substrate or production of TWIK-8 inhibitors. In addition, the TWIK-8 proteins can be used to screen for naturally occurring TWIK-8 substrates, to screen for drugs or compounds which modulate TWIK-8 activity, as well as to treat disorders characterized by insufficient or excessive production of TWIK-8 protein or production of TWIK-8 protein forms which have decreased, aberrant or unwanted activity compared to TWIK-8 wild type protein, *e.g.*, potassium channel associated disorders. Such disorders include CNS disorders such as cognitive and neurodegenerative disorders (*e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, senile dementia, Huntington's disease, Gilles de la Tourette's syndrome, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, AIDS related dementia, and Jakob-Creutzfeldt disease; autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders, such as depression, schizophrenia, schizoaffective disorder, korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss, attention deficit disorder, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, phobias, panic disorder, and bipolar affective disorder (*e.g.*, severe bipolar affective (mood) disorder (BP-1) and bipolar affective neurological disorders

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(*e.g.*, migraine and obesity)); cardiac disorders (*e.g.*, arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, Jervell syndrome, Lange-Nielsen syndrome, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, and arrhythmia), muscular disorders (*e.g.*, paralysis, muscle weakness (*e.g.*, ataxia, myotonia, and myokymia), muscular dystrophy (*e.g.*, Duchenne muscular dystrophy or myotonic dystrophy), spinal muscular atrophy, congenital myopathies, central core disease, rod myopathy, central nuclear myopathy, Lambert-Eaton syndrome, denervation, and infantile spinal muscular atrophy (Werdnig-Hoffman disease), pain disorders (*e.g.*, headache (*e.g.*, tension headache or migraine), back pain, cancer pain, arthritis pain, or neurogenic pain), and disorders of cellular growth, differentiation, or migration (*e.g.*, cancer, *e.g.*, carcinoma, sarcoma, or leukemia; tumor angiogenesis and metastasis; skeletal dysplasia; neuronal deficiencies resulting from impaired neural induction and patterning; and hematopoietic and/or myeloproliferative disorders. Moreover, the anti-TWIK-8 antibodies of the invention can be used to detect and isolate TWIK-8 proteins, to regulate the bioavailability of TWIK-8 proteins, and to modulate TWIK-8 activity.

A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to TWIK-8 proteins, have a stimulatory or inhibitory effect on, for example, TWIK-8 expression or TWIK-8 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of TWIK-8 substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a TWIK-8 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for

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screening candidate or test compounds which bind to or modulate the activity of a TWIK-8 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological
5 libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds
10 (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in
15 Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP
20 '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which
25 expresses a TWIK-8 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate TWIK-8 activity is determined. Determining the ability of the test compound to modulate TWIK-8 activity can be accomplished by monitoring, for example, the release of a neurotransmitter from a cell which expresses TWIK-8. The cell, for example, can be of mammalian origin,
30 *e.g.*, a neuronal cell, cardiac cell or a skin cell.

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The ability of the test compound to modulate TWIK-8 binding to a substrate or to bind to TWIK-8 can also be determined. Determining the ability of the test compound to modulate TWIK-8 binding to a substrate can be accomplished, for example, by coupling the TWIK-8 substrate with a radioisotope or enzymatic label such that binding of the TWIK-8 substrate to TWIK-8 can be determined by detecting the labeled TWIK-8 substrate in a complex. Alternatively, TWIK-8 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate TWIK-8 binding to a TWIK-8 substrate in a complex. Determining the ability of the test compound to bind TWIK-8 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to TWIK-8 can be determined by detecting the labeled TWIK-8 compound in a complex. For example, compounds (*e.g.*, TWIK-8 substrates) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, a TWIK-8 substrate) to interact with TWIK-8 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with TWIK-8 without the labeling of either the compound or the TWIK-8. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and TWIK-8.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a TWIK-8 target molecule (*e.g.*, a TWIK-8 substrate) with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the TWIK-8 target molecule. Determining the ability of the test compound to modulate the activity of a TWIK-8 target molecule can be

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accomplished, for example, by determining the ability of the TWIK-8 protein to bind to or interact with the TWIK-8 target molecule.

Determining the ability of the TWIK-8 protein, or a biologically active fragment thereof, to bind to or interact with a TWIK-8 target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the TWIK-8 protein to bind to or interact with a TWIK-8 target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular response (*i.e.*, changes in intracellular K^+ levels), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response.

In yet another embodiment, an assay of the present invention is a cell-free assay in which a TWIK-8 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the TWIK-8 protein or biologically active portion thereof is determined. Preferred biologically active portions of the TWIK-8 proteins to be used in assays of the present invention include fragments which participate in interactions with non-TWIK-8 molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the TWIK-8 protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the TWIK-8 protein or biologically active portion thereof with a known compound which binds TWIK-8 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a TWIK-8 protein, wherein determining the ability of the test compound to interact with a TWIK-8 protein comprises determining the ability of the test compound to preferentially bind to TWIK-8 or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a TWIK-8 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the TWIK-8 protein or biologically active portion thereof is determined. Determining the ability of

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the test compound to modulate the activity of a TWIK-8 protein can be accomplished, for example, by determining the ability of the TWIK-8 protein to bind to a TWIK-8 target molecule by one of the methods described above for determining direct binding. Determining the ability of the TWIK-8 protein to bind to a TWIK-8 target molecule can
5 also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon
10 resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a TWIK-8 protein can be accomplished by determining the ability of the TWIK-8 protein to further modulate the activity of a downstream effector
15 of a TWIK-8 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a TWIK-8 protein or biologically active portion thereof with a known compound which binds the
20 TWIK-8 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the TWIK-8 protein, wherein determining the ability of the test compound to interact with the TWIK-8 protein comprises determining the ability of the TWIK-8 protein to preferentially bind to or modulate the activity of a TWIK-8 target molecule.

25 In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either TWIK-8 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a TWIK-8 protein, or interaction of a TWIK-8 protein with a target
30 molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion

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protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/TWIK-8 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre
5 plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or TWIK-8 protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex
10 determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of TWIK-8 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a TWIK-8 protein or a TWIK-8
15 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated TWIK-8 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with TWIK-8 protein or
20 target molecules but which do not interfere with binding of the TWIK-8 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or TWIK-8 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the
25 TWIK-8 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the TWIK-8 protein or target molecule.

In another embodiment, modulators of TWIK-8 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of TWIK-8 mRNA or protein in the cell is determined. The level of expression of TWIK-8
30 mRNA or protein in the presence of the candidate compound is compared to the level of expression of TWIK-8 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of TWIK-8 expression

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based on this comparison. For example, when expression of TWIK-8 mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of TWIK-8 mRNA or protein expression. Alternatively, when expression of TWIK-8 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of TWIK-8 mRNA or protein expression. The level of TWIK-8 mRNA or protein expression in the cells can be determined by methods described herein for detecting TWIK-8 mRNA or protein.

10 In yet another aspect of the invention, the TWIK-8 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind
15 to or interact with TWIK-8 ("TWIK-8-binding proteins" or "TWIK-8-bp") and are involved in TWIK-8 activity. Such TWIK-8-binding proteins are also likely to be involved in the propagation of signals by the TWIK-8 proteins or TWIK-8 targets as, for example, downstream elements of a TWIK-8-mediated signaling pathway. Alternatively, such TWIK-8-binding proteins are likely to be TWIK-8 inhibitors.

20 The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a TWIK-8 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a
25 library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a TWIK-8-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene
30 (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell

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colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the TWIK-8 protein.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a TWIK-8 protein can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for pain.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a TWIK-8 modulating agent, an antisense TWIK-8 nucleic acid molecule, a TWIK-8-specific antibody, or a TWIK-8-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the TWIK-8 nucleotide sequences, described herein, can be used to map the location of the TWIK-8

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genes on a chromosome. The mapping of the TWIK-8 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, TWIK-8 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the TWIK-8 nucleotide sequences.

- 5 Computer analysis of the TWIK-8 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the TWIK-8 sequences will yield an amplified fragment.

- 10 Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains
15 the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell
20 hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

- PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the TWIK-8 nucleotide sequences
25 to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a TWIK-8 sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to
30 chromosome specific cDNA libraries.

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Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle.

5 The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity

10 for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single

15 chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

20 Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can

25 then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the TWIK-8 gene can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected

30 individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are

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visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

5 2. Tissue Typing

 The TWIK-8 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is
10 digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

15 Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the TWIK-8 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and
20 subsequently sequence it.

 Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals
25 and from tissue. The TWIK-8 nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some
30 degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The

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noncoding sequences of SEQ ID NO:1 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual
5 identification would be 500-2,000.

If a panel of reagents from TWIK-8 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from
10 extremely small tissue samples.

3. Use of TWIK-8 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator
15 of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the
20 origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular
25 individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique.
30 Examples of polynucleotide reagents include the TWIK-8 nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases.

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The TWIK-8 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a
5 tissue of unknown origin. Panels of such TWIK-8 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, TWIK-8 primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

10

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically.
15 Accordingly, one aspect of the present invention relates to diagnostic assays for determining TWIK-8 protein and/or nucleic acid expression as well as TWIK-8 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant or unwanted TWIK-8 expression or
20 activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with TWIK-8 protein, nucleic acid expression or activity. For example, mutations in a TWIK-8 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to
25 the onset of a disorder characterized by or associated with TWIK-8 protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of TWIK-8 in clinical trials.

These and other agents are described in further detail in the following sections.

30

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of TWIK-8 protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of
5 detecting TWIK-8 protein or nucleic acid (*e.g.*, mRNA, or genomic DNA) that encodes TWIK-8 protein such that the presence of TWIK-8 protein or nucleic acid is detected in the biological sample. A preferred agent for detecting TWIK-8 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to TWIK-8 mRNA or genomic DNA. The nucleic acid probe can be, for example, the TWIK-8 nucleic acid
10 set forth in SEQ ID NO:1 or 3, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to TWIK-8 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

15 A preferred agent for detecting TWIK-8 protein is an antibody capable of binding to TWIK-8 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by
20 coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.
25 The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect TWIK-8 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of TWIK-8 mRNA include Northern hybridizations and *in situ*
30 hybridizations. *In vitro* techniques for detection of TWIK-8 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of TWIK-8 genomic DNA

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include Southern hybridizations. Furthermore, *in vivo* techniques for detection of TWIK-8 protein include introducing into a subject a labeled anti-TWIK-8 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

5 In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

 In another embodiment, the methods further involve obtaining a control
10 biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting TWIK-8 protein, mRNA, or genomic DNA, such that the presence of TWIK-8 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of TWIK-8 protein, mRNA or genomic DNA in the control sample with the presence of TWIK-8 protein, mRNA or genomic
15 DNA in the test sample.

 The invention also encompasses kits for detecting the presence of TWIK-8 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting TWIK-8 protein or mRNA in a biological sample; means for determining the amount of TWIK-8 in the sample; and means for comparing the amount
20 of TWIK-8 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect TWIK-8 protein or nucleic acid.

2. Prognostic Assays

25 The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted TWIK-8 expression or activity. As used herein, the term "aberrant" includes a TWIK-8 expression or activity which deviates from the wild type TWIK-8 expression or activity. Aberrant expression or activity includes increased or decreased expression or
30 activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant TWIK-8 expression or activity is intended to include the cases in

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which a mutation in the TWIK-8 gene causes the TWIK-8 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional TWIK-8 protein or a protein which does not function in a wild-type fashion, *e.g.*, a protein which does not interact with a TWIK-8 substrate, *e.g.*, a non-potassium channel subunit or ligand, or one which interacts with a non-TWIK-8 substrate, *e.g.* a non-potassium channel subunit or ligand. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as cellular proliferation. For example, the term unwanted includes a TWIK-8 expression or activity which is undesirable in a subject.

10 The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in TWIK-8 protein activity or nucleic acid expression, such as a CNS disorder (*e.g.*, a cognitive or neurodegenerative disorder, or a pain disorder), a muscular disorder, a cellular proliferation, growth, differentiation, or migration disorder, or a cardiovascular disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in TWIK-8 protein activity or nucleic acid expression, such as a CNS disorder, a cardiovascular disorder, a pain disorder, or a cellular proliferation, growth, differentiation, or migration disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant or unwanted TWIK-8 expression or activity in which a test sample is obtained from a subject and TWIK-8 protein or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of TWIK-8 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted TWIK-8 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, cerebrospinal fluid or serum), cell sample, or tissue.

 Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted TWIK-8 expression or activity. For example, such methods can be used to determine whether a subject can

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be effectively treated with an agent for a CNS disorder, a muscular disorder, a pain disorder, a cardiovascular, or a cellular proliferation, growth, differentiation, or migration disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant or unwanted TWIK-8 expression or activity in which a test sample is obtained and TWIK-8 protein or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of TWIK-8 protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted TWIK-8 expression or activity).

10 The methods of the invention can also be used to detect genetic alterations in a TWIK-8 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in TWIK-8 protein activity or nucleic acid expression, such as a CNS disorder, a cardiovascular disorder, a pain disorder, or a disorder of cellular growth, differentiation, or migration. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a TWIK-8 -protein, or the mis-expression of the TWIK-8 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a TWIK-8 gene; 2) an addition of one or more nucleotides to a TWIK-8 gene; 3) a substitution of one or more nucleotides of a TWIK-8 gene, 4) a chromosomal rearrangement of a TWIK-8 gene; 5) an alteration in the level of a messenger RNA transcript of a TWIK-8 gene, 6) aberrant modification of a TWIK-8 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a TWIK-8 gene, 8) a non-wild type level of a TWIK-8-protein, 9) allelic loss of a TWIK-8 gene, and 10) inappropriate post-translational modification of a TWIK-8-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a TWIK-8 gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

30 In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a

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ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the TWIK-8-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the
5 steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a TWIK-8 gene under conditions such that hybridization and amplification of the TWIK-8-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of
10 the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional
15 amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such
20 molecules are present in very low numbers.

In an alternative embodiment, mutations in a TWIK-8 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel
25 electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

30 In other embodiments, genetic mutations in TWIK-8 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.*

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(1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in TWIK-8 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the TWIK-8 gene and detect mutations by comparing the sequence of the sample TWIK-8 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the TWIK-8 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type TWIK-8 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In

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other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation.

- 5 See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called
10 "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in TWIK-8 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a TWIK-8
15 sequence, *e.g.*, a wild-type TWIK-8 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to
20 identify mutations in TWIK-8 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and
25 control TWIK-8 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary
30 structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex

molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing
5 gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control
10 and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions
15 which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele-specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

20 Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3'
25 end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification
30 (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect

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the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a TWIK-8 gene.

Furthermore, any cell type or tissue in which TWIK-8 is expressed may be utilized in the prognostic assays described herein.

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3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a TWIK-8 protein (*e.g.*, the modulation of pain signaling mechanisms, neurotransmitter release and/or membrane excitability) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase TWIK-8 gene expression, protein levels, or upregulate TWIK-8 activity, can be monitored in clinical trials of subjects exhibiting decreased TWIK-8 gene expression, protein levels, or downregulated TWIK-8 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease TWIK-8 gene expression, protein levels, or downregulate TWIK-8 activity, can be monitored in clinical trials of subjects exhibiting increased TWIK-8 gene expression, protein levels, or upregulated TWIK-8 activity. In such clinical trials, the expression or activity of a TWIK-8 gene, and preferably, other genes that have been implicated in, for example, a TWIK-8-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including TWIK-8, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates TWIK-8 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on TWIK-8-associated disorders (*e.g.*, disorders characterized by deregulated pain signaling mechanisms, neurotransmitter release and/or membrane excitability), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of

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TWIK-8 and other genes implicated in the TWIK-8-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of TWIK-8 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a TWIK-8 protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the TWIK-8 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the TWIK-8 protein, mRNA, or genomic DNA in the pre-administration sample with the TWIK-8 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of TWIK-8 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of TWIK-8 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, TWIK-8 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted TWIK-8 expression or activity, *e.g.* a potassium channel

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associated disorder such as a CNS disorder, a cardiovascular disorder, a pain disorder, or a cellular proliferation, growth, differentiation, or migration disorder. "Treatment", as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, the symptoms of disease or disorder or the predisposition toward a disease or disorder. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

"Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the TWIK-8 molecules of the present invention or TWIK-8 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted TWIK-8 expression or activity, by administering to the subject a TWIK-8 or an agent which modulates TWIK-8 expression or at least one TWIK-8 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted TWIK-8 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the

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manifestation of symptoms characteristic of the TWIK-8 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of TWIK-8 aberrancy, for example, a TWIK-8, TWIK-8 agonist or TWIK-8 antagonist agent can be used for treating the subject. The appropriate agent can be
5 determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating TWIK-8 expression or activity for therapeutic purposes. Accordingly, in an exemplary
10 embodiment, the modulatory method of the invention involves contacting a cell with a TWIK-8 or agent that modulates one or more of the activities of TWIK-8 protein activity associated with the cell. An agent that modulates TWIK-8 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a TWIK-8 protein (*e.g.*, a TWIK-8 substrate), a TWIK-8 antibody, a
15 TWIK-8 agonist or antagonist, a peptidomimetic of a TWIK-8 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more TWIK-8 activities. Examples of such stimulatory agents include active TWIK-8 protein and a nucleic acid molecule encoding TWIK-8 that has been introduced into the cell. In another embodiment, the agent inhibits one or more TWIK-8 activities. Examples of
20 such inhibitory agents include antisense TWIK-8 nucleic acid molecules, anti-TWIK-8 antibodies, and TWIK-8 inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or
25 unwanted expression or activity of a TWIK-8 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) TWIK-8 expression or activity. In another embodiment, the method involves administering a TWIK-8 protein or nucleic acid molecule as
30 therapy to compensate for reduced, aberrant, or unwanted TWIK-8 expression or activity.

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Stimulation of TWIK-8 activity is desirable in situations in which TWIK-8 is abnormally downregulated and/or in which increased TWIK-8 activity is likely to have a beneficial effect. Likewise, inhibition of TWIK-8 activity is desirable in situations in which TWIK-8 is abnormally upregulated and/or in which decreased TWIK-8 activity is likely to have a beneficial effect.

3. Pharmacogenomics

The TWIK-8 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on TWIK-8 activity (*e.g.*, TWIK-8 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) TWIK-8-associated disorders (*e.g.*, pain disorders, cardiovascular disorders, proliferative disorders) associated with aberrant or unwanted TWIK-8 activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a TWIK-8 molecule or TWIK-8 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a TWIK-8 molecule or TWIK-8 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited

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enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (*e.g.*, a TWIK-8 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated

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drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic
5 and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-
10 formed metabolite morphine. The other extreme are the so-called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an
15 animal dosed with a drug (*e.g.*, a TWIK-8 molecule or TWIK-8 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for
20 prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a TWIK-8 molecule or TWIK-8 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

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VI. Electronic Apparatus Readable Media and Arrays

Electronic apparatus readable media comprising TWIK-8 sequence information is also provided. As used herein, "TWIK-8 sequence information" refers to any nucleotide and/or amino acid sequence information particular to the TWIK-8 molecules
30 of the present invention, including but not limited to full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequences, and the like.

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Moreover, information “related to” said TWIK-8 sequence information includes detection of the presence or absence of a sequence (*e.g.*, detection of expression of a sequence, fragment, polymorphism, etc.), determination of the level of a sequence (*e.g.*, detection of a level of expression, for example, a quantitative detection), detection of a reactivity to a sequence (*e.g.*, detection of protein expression and/or levels, for example, using a sequence-specific antibody), and the like. As used herein, “electronic apparatus readable media” refers to any suitable medium for storing, holding or containing data or information that can be read and accessed directly by an electronic apparatus. Such media can include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as compact disc; electronic storage media such as RAM, ROM, EPROM, EEPROM and the like; general hard disks and hybrids of these categories such as magnetic/optical storage media. The medium is adapted or configured for having recorded thereon TWIK-8 sequence information of the present invention.

As used herein, the term “electronic apparatus” is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the present invention include stand-alone computing apparatus; networks, including a local area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as a personal digital assistants (PDAs), cellular phone, pager and the like; and local and distributed processing systems.

As used herein, “recorded” refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising the TWIK-8 sequence information.

A variety of software programs and formats can be used to store the sequence information on the electronic apparatus readable medium. For example, the sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like, as well as in other forms. Any number of data processor

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structuring formats (*e.g.*, text file or database) may be employed in order to obtain or create a medium having recorded thereon the TWIK-8 sequence information.

By providing TWIK-8 sequence information in readable form, one can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the sequence information in readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

The present invention therefore provides a medium for holding instructions for performing a method for determining whether a subject has a TWIK-8- associated disease or disorder or a pre-disposition to a TWIK-8-associated disease or disorder, wherein the method comprises the steps of determining TWIK-8 sequence information associated with the subject and based on the TWIK-8 sequence information, determining whether the subject has a TWIK-8 -associated disease or disorder or a pre-disposition to a TWIK-8-associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a TWIK-8-associated disease or disorder or a pre-disposition to a disease associated with a TWIK-8 wherein the method comprises the steps of determining TWIK-8 sequence information associated with the subject, and based on the TWIK-8 sequence information, determining whether the subject has a TWIK-8 -associated disease or disorder or a pre-disposition to a TWIK-8-associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. The method may further comprise the step of receiving phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

The present invention also provides in a network, a method for determining whether a subject has a TWIK-8-associated disease or disorder or a pre-disposition to a TWIK-8 associated disease or disorder associated with TWIK-8, said method comprising the steps of receiving TWIK-8 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to TWIK-8 and/or a

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TWIK-8-associated disease or disorder, and based on one or more of the phenotypic information, the TWIK-8 information (*e.g.*, sequence information and/or information related thereto), and the acquired information, determining whether the subject has a TWIK-8-associated disease or disorder or a pre-disposition to a TWIK-8-associated
5 disease or disorder (*e.g.*, a pain disorder, a cardiovascular disorder, a CNS disorder, or a cellular proliferation, growth, differentiation, or migration disorder). The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a business method for determining whether a
10 subject has a TWIK-8-associated disease or disorder or a pre-disposition to a TWIK-8-associated disease or disorder, said method comprising the steps of receiving information related to TWIK-8 (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to TWIK-8 and/or related to a TWIK-8-associated
15 disease or disorder, and based on one or more of the phenotypic information, the TWIK-8 information, and the acquired information, determining whether the subject has a TWIK-8-associated disease or disorder or a pre-disposition to a TWIK-8-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

20 The invention also includes an array comprising a TWIK-8 sequence of the present invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be TWIK-8. This
25 allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be
30 grouped on the basis of their tissue expression *per se* and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene

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expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of a TWIK-8-associated disease or disorder, progression of TWIK-8-associated disease or disorder, and processes, such as cellular transformation associated with the TWIK-8-associated disease or disorder.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (*e.g.*, ascertaining the effect of TWIK-8 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (*e.g.*, including TWIK-8) that could serve as a molecular target for diagnosis or therapeutic intervention.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

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EXAMPLES**EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF HUMAN
TWIK-8 cDNA**

5

In this example, the identification and characterization of the gene encoding human TWIK-8 (clone Fbh12303) is described.

Isolation of the TWIK-8 cDNA

10 The invention is based, at least in part, on the discovery of a human gene encoding a novel protein, referred to herein as TWIK-8. The entire sequence of the human clone Fbh12303 was determined and found to contain an open reading frame termed human "TWIK-8."

The nucleotide sequence encoding the human TWIK-8 protein is shown in
15 Figure 1 and is set forth as SEQ ID NO:1. The protein encoded by this nucleic acid comprises about 419 amino acids and has the amino acid sequence shown in Figure 1 and set forth as SEQ ID NO:2. The coding region (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3. Clone Fbh12303, comprising the coding region of human TWIK-8, was deposited with the American Type Culture Collection (ATCC®),
20 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

Analysis of the Human TWIK-8 Molecule

The amino acid sequence of human TWIK-8 was analyzed using the program
25 PSORT (<http://www.psорт.nibb.ac.jp>) to predict the localization of the proteins within the cell. This program assesses the presence of different targeting and localization amino acid sequences within the query sequence. The results of the analysis show that human TWIK-8 (SEQ ID NO:2) may be localized to the endoplasmic reticulum or to the mitochondrion.

30 An analysis of the amino acid sequence of human TWIK-8 using the Signal P program (Henrik, *et al.* (1997) *Protein Engineering* 10:1-6), identified the presence of a signal peptide from amino acids 1-46 (Figure 2).

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A search was performed against the Memsat database (Figure 3) resulting in the identification of six transmembrane domains in the amino acid sequence of the native human TWIK-8 (SEQ ID NO: 2) at about residues 32-50, 116-137, 144-165, 195-219, 226-242, and 260-283. This search further identified five transmembrane domains in the amino acid sequence of the predicted mature form of this protein, at about residues 70-91, 98-119, 149-173, 180-196, and 214-237. The results of this search are set forth in Figure 3.

A search was performed against the HMM database (Figure 4), resulting in the identification of a "seven-transmembrane receptor domain" from about residues 25-244, and a "cyclic nucleotide-gated channel domain" from about residues 27-204 in the amino acid sequence of human TWIK-8 (SEQ ID NO:2).

A search was also performed against the ProDom database (Figure 5), resulting in the identification of "TRAAK potassium channel domains" from about residues 50-104 (score= 175), 175-199 (score = 115), and 288-382 (score = 135); a "potassium channel protein domain" from about residues 99-153 (score = 101); a "voltage-gated potassium channel domain" from about residues 102-168 (score = 115); an "outward-rectifier TOK1 potassium channel domain" from about residues 215-270 (score = 70); and a "potassium channel subunit domain" from about residues 216-287 (score = 156) in the amino acid sequence of human TWIK-8 (SEQ ID NO:2).

A BLASTX 2.0 search against the NRP/protot database, using a score of 100, a wordlength of 3, and a Blosum 62 matrix (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403), of the translated nucleotide sequence of human TWIK-8 revealed that human TWIK-8 has limited sequence homology to *Mus musculus* TRAAK K⁺ channel subunit mRNA (Accession Number AF056492), to *Homo sapiens* TREK-1 potassium channel (KCNK2) mRNA (Accession Number AF129399), to *Mus musculus* TREK-1 K⁺ channel subunit mRNA (Accession Number U73488), and to *Homo sapiens* two-pore potassium channel TPKC1 mRNA (Accession number AF004711).

Tissue Distribution of TWIK-8 mRNA by PCR and *In Situ* Hybridization

The tissue distribution of TWIK-8 mRNA is determined by Polymerase Chain Reaction (PCR) on cDNA libraries of normal human tissues using oligonucleotide primers based on the human TWIK-8 sequence.

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The tissue distribution of TWIK-8 mRNA is also determined using *in situ* hybridization. For *in situ* analysis, various tissues, e.g. tissues obtained from brain, are first frozen on dry ice. Ten-micrometer-thick sections of the tissues are postfixed with 4% formaldehyde in DEPC treated 1X phosphate- buffered saline at room temperature for 10 minutes before being rinsed twice in DEPC 1X phosphate-buffered saline and
5 once in 0.1 M triethanolamine-HCl (pH 8.0). Following incubation in 0.25% acetic anhydride-0.1 M triethanolamine-HCl for 10 minutes, sections are rinsed in DEPC 2X SSC (1X SSC is 0.15M NaCl plus 0.015M sodium citrate). Tissue is then dehydrated through a series of ethanol washes, incubated in 100% chloroform for 5 minutes, and
10 then rinsed in 100% ethanol for 1 minute and 95% ethanol for 1 minute and allowed to air dry.

Hybridizations are performed with ^{35}S -radiolabeled (5×10^7 cpm/ml) cRNA probes. Probes are incubated in the presence of a solution containing 600 mM NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 0.01% sheared salmon sperm DNA, 0.01% yeast
15 tRNA, 0.05% yeast total RNA type X1, 1X Denhardt's solution, 50% formamide, 10% dextran sulfate, 100 mM dithiothreitol, 0.1% sodium dodecyl sulfate (SDS), and 0.1% sodium thiosulfate for 18 hours at 55°C.

After hybridization, slides are washed with 2X SSC. Sections are then sequentially incubated at 37°C in TNE (a solution containing 10 mM Tris-HCl (pH 7.6),
20 500 mM NaCl, and 1 mM EDTA), for 10 minutes, in TNE with 10µg of RNase A per ml for 30 minutes, and finally in TNE for 10 minutes. Slides are then rinsed with 2X SSC at room temperature, washed with 2X SSC at 50°C for 1 hour, washed with 0.2X SSC at 55°C for 1 hour, and 0.2X SSC at 60°C for 1 hour. Sections are then dehydrated rapidly through serial ethanol-0.3 M sodium acetate concentrations before being air
25 dried and exposed to Kodak Biomax MR scientific imaging film for 24 hours and subsequently dipped in NB-2 photoemulsion and exposed at 4°C for 7 days before being developed and counter stained.

Tissue Distribution of human TWIK-8 mRNA using Taqman™ analysis

30 This example describes the tissue distribution of human TWIK-8 mRNA in a variety of cells and tissues, as determined using the TaqMan™ procedure. The Taqman™ procedure is a quantitative, reverse transcription PCR-based approach for

- 100 -

detecting mRNA. The RT-PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold™ DNA Polymerase to cleave a TaqMan™ probe during PCR. Briefly, cDNA was generated from the samples of interest, *e.g.*, various human tissue samples, and used as the starting material for PCR amplification. In addition to the 5' and 3' gene-specific
5 primers, a gene-specific oligonucleotide probe (complementary to the region being amplified) was included in the reaction (*i.e.*, the Taqman™ probe). The TaqMan™ probe includes the oligonucleotide with a fluorescent reporter dye covalently linked to the 5' end of the probe (such as FAM (6-carboxyfluorescein), TET (6-carboxy-4,7,2',7'-tetrachlorofluorescein), JOE (6-carboxy-4,5-dichloro-2,7-dimethoxyfluorescein), or
10 VIC) and a quencher dye (TAMRA (6-carboxy-N,N,N',N'-tetramethylrhodamine) at the 3' end of the probe.

During the PCR reaction, cleavage of the probe separates the reporter dye and the quencher dye, resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the
15 reporter dye. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'-3' nucleolytic activity of the AmpliTaq™ Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the
20 target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized
25 using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control gene confirms efficient removal of genomic DNA contamination.

Highest expression of TWIK-8 mRNA was detected in cortex, followed by dorsal root ganglia, and hypothalamus. Weak expression was also detected in erythroid
30 tissue, followed by HUVEC, spinal cord, hemangioma, kidney, normal ovary and ovary tumor, megakaryocytes, normal prostate and prostate tumor. Weak expression was also detected in breast tumor although no expression was detected in normal breast tissue.

**EXAMPLE 2: EXPRESSION OF RECOMBINANT TWIK-8 PROTEIN IN
BACTERIAL CELLS**

5

In this example, TWIK-8 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, TWIK-8 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-TWIK-8 fusion protein
10 in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

15

**EXAMPLE 3: EXPRESSION OF RECOMBINANT TWIK-8
PROTEIN IN COS CELLS**

To express the TWIK-8 gene in COS cells, the pcDNA/Amp vector by
20 Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire TWIK-8 protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the
25 polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the TWIK-8 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the TWIK-8 coding sequence starting from the
30 initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the TWIK-8 coding sequence. The PCR amplified fragment and the

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pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the TWIK-8 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 α , SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the TWIK-8-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the TWIK-8 polypeptide is detected by radiolabelling (^{35}S -methionine or ^{35}S -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with ^{35}S -methionine (or ^{35}S -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the TWIK-8 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the TWIK-8 polypeptide is detected by radiolabelling and immunoprecipitation using a TWIK-8 specific monoclonal antibody.

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Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

5 claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:
 - (a) a nucleic acid molecule comprising the nucleotide sequence set forth
5 in SEQ ID NO:1; and
 - (b) a nucleic acid molecule comprising the nucleotide sequence set forth
in SEQ ID NO:3.
2. An isolated nucleic acid molecule which encodes a polypeptide
10 comprising the amino acid sequence set forth in SEQ ID NO: 2.
3. An isolated nucleic acid molecule comprising the nucleotide sequence
contained in the plasmid deposited with ATCC® as Accession Number _____.
- 15 4. An isolated nucleic acid molecule which encodes a naturally occurring
allelic variant of a polypeptide comprising the amino acid sequence set forth in SEQ ID
NO: 2.
5. An isolated nucleic acid molecule selected from the group consisting of:
 - 20 a) a nucleic acid molecule comprising a nucleotide sequence which
is at least 60% identical to the nucleotide sequence of SEQ ID NO:1 or 3, or a
complement thereof;
 - b) a nucleic acid molecule comprising a fragment of at least 50
nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3,
25 or a complement thereof;
 - c) a nucleic acid molecule which encodes a polypeptide comprising
an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ
ID NO:2; and
 - d) a nucleic acid molecule which encodes a fragment of a
30 polypeptide comprising the amino acid sequence of SEQ ID NO: 2, wherein the
fragment comprises at least 15 contiguous amino acid residues of the amino acid
sequence of SEQ ID NO: 2.

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6. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 under stringent conditions.
7. An isolated nucleic acid molecule comprising a nucleotide sequence
5 which is complementary to the nucleotide sequence of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5.
8. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5, and a nucleotide sequence encoding a heterologous
10 polypeptide.
9. A vector comprising the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5.
- 15 10. The vector of claim 9, which is an expression vector.
11. A host cell transfected with the expression vector of claim 10.
12. A method of producing a polypeptide comprising culturing the host cell
20 of claim 11 in an appropriate culture medium to, thereby, produce the polypeptide.
13. An isolated polypeptide selected from the group consisting of:
 - a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, wherein the fragment comprises at least 15 contiguous amino acids of
25 SEQ ID NO: 2;
 - b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule consisting of SEQ ID NO:1 or 3 under stringent conditions;

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- c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60 % identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3;
- d) a polypeptide comprising an amino acid sequence which is at
5 least 60% identical to the amino acid sequence of SEQ ID NO: 2.

14. The isolated polypeptide of claim 13 comprising the amino acid sequence of SEQ ID NO:2.

- 10 15. The polypeptide of claim 13, further comprising heterologous amino acid sequences.

16. An antibody which selectively binds to a polypeptide of claim 13.

- 15 17. A method for detecting the presence of a polypeptide of claim 13 in a sample comprising:

- a) contacting the sample with a compound which selectively binds to the polypeptide; and
- b) determining whether the compound binds to the polypeptide in
20 the sample to thereby detect the presence of a polypeptide of claim 13 in the sample.

18. The method of claim 17, wherein the compound which binds to the polypeptide is an antibody.

- 25 19. A kit comprising a compound which selectively binds to a polypeptide of claim 13 and instructions for use.

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20. A method for detecting the presence of a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

5 b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in the sample.

21. The method of claim 20, wherein the sample comprises mRNA
10 molecules and is contacted with a nucleic acid probe.

22. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 and instructions for use.

15 23. A method for identifying a compound which binds to a polypeptide of claim 13 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

b) determining whether the polypeptide binds to the test compound.
20

24. The method of claim 23, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test compound/polypeptide binding;

25 b) detection of binding using a competition binding assay; and

c) detection of binding using an assay for TWIK-8 activity.

25. A method for modulating the activity of a polypeptide of claim 13 comprising contacting the polypeptide or a cell expressing the polypeptide with a
30 compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

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26. A method for identifying a compound which modulates the activity of a polypeptide of claim 13 comprising:

- a) contacting a polypeptide of claim 13 with a test compound; and
 - b) determining the effect of the test compound on the activity of the
- 5 polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

Input file Fbh12303FL.seq; Output File 12303.trans
Sequence length 1408

CCACGCGTCCGCGGACGCGGTGGGTCTGGCACCAGGCGAGGCCGTGCTGGAACCCGGGCCCTCAGCCGCAGCCGCAGCGGGGC

M	T	T	A	P	Q	E	P	P	A	R	P	L	Q	A	G	S	G		18	
CGAC	ATG	ACG	ACA	GCT	CCC	CAG	GAG	CCC	CCC	GCC	CGG	CCC	CTC	CAG	GCG	GGC	AGT	GGA	54	
A	G	P	A	P	G	R	A	M	R	S	T	T	L	L	A	L	L	A	L	38
GCT	GGC	CCG	GCG	CCT	GGG	CGC	GCC	ATG	CGC	AGC	ACC	ACG	CTC	CTG	GCC	CTG	CTG	GCG	CTG	114
V	L	L	Y	L	V	S	G	A	L	V	F	R	A	L	E	Q	P	H	E	58
GTC	TTG	CTT	TAC	TTG	GTG	TCT	GGT	GCC	CTG	GTG	TTC	CGG	GCC	CTG	GAG	CAG	CCC	CAC	GAG	174
Q	Q	A	Q	R	E	L	G	E	V	R	E	K	F	L	R	A	H	P	C	78
CAG	CAG	GCC	CAG	AGG	GAG	CTG	GGG	GAG	GTC	CGA	GAG	AAG	TTC	CTG	AGG	GCC	CAT	CCG	TGT	234
V	S	D	Q	E	L	G	L	L	I	K	E	V	A	D	A	L	G	G	G	98
GTG	AGC	GAC	CAG	GAG	CTG	GGC	CTC	CTC	ATC	AAG	GAG	GTG	GCT	GAT	GCC	CTG	GGA	GGG	GGT	294
A	D	P	E	T	N	S	T	S	N	S	S	H	S	A	W	D	L	G	S	118
GCG	GAC	CCA	GAA	ACC	AAC	TCG	ACC	AGC	AAC	AGC	AGC	CAC	TCA	GCC	TGG	GAC	CTG	GGC	AGC	354
A	F	F	F	S	G	T	I	I	T	T	I	G	Y	G	N	V	A	L	R	138
GCC	TTC	TTT	TTC	TCA	GGG	ACC	ATC	ATC	ACC	ACC	ATC	GGC	TAT	GGC	AAT	GTG	GCC	CTG	CGC	414
T	D	A	G	R	L	F	C	I	F	Y	A	L	V	G	I	P	L	F	G	158
ACA	GAT	GCC	GGG	CGC	CTC	TTC	TGC	ATC	TTT	TAT	GCG	CTG	GTG	GGG	ATT	CCG	CTG	TTT	GGG	474
I	L	L	A	G	V	G	D	R	L	G	S	S	L	R	H	G	I	G	H	178
ATC	CTA	CTG	GCA	GGG	GTC	GGG	GAC	CGG	CTG	GGC	TCC	TCC	CTG	CGC	CAT	GGC	ATC	GGT	CAC	534
I	E	A	I	F	L	K	W	H	V	P	P	E	L	V	R	V	L	S	A	198
ATT	GAA	GCC	ATC	TTC	TTG	AAG	TGG	CAC	GTG	CCA	CCG	GAG	CTA	GTA	AGA	GTG	CTG	TCG	GCG	594
M	L	F	L	L	I	G	C	L	L	F	V	L	T	P	T	F	V	F	C	218
ATG	CTT	TTC	CTG	CTG	ATC	GGC	TGC	CTG	CTC	TTT	GTC	CTC	ACG	CCC	ACG	TTC	GTG	TTC	TGC	654
Y	M	E	D	W	S	K	L	E	A	I	Y	F	V	I	V	T	L	T	T	238
TAT	ATG	GAG	GAC	TGG	AGC	AAG	CTG	GAG	GCC	ATC	TAC	TTT	GTC	ATA	GTG	ACG	CTT	ACC	ACC	714
V	G	F	G	D	Y	V	A	G	A	D	P	R	Q	D	S	P	A	Y	Q	258
GTG	GGC	TTT	GGC	GAC	TAT	GTG	GCC	GGC	GCG	GAC	CCC	AGG	CAG	GAC	TCC	CCG	GCC	TAT	CAG	774
P	L	V	W	F	W	I	L	L	G	L	A	Y	F	A	S	V	L	T	T	278
CCG	CTG	GTG	TGG	TTC	TGG	ATC	CTG	CTC	GGC	CTG	GCT	TAC	TTC	GCC	TCA	GTG	CTC	ACC	ACC	834
I	G	N	W	L	R	V	V	S	R	R	T	R	A	E	M	G	G	L	T	298
ATC	GGG	AAC	TGG	CTG	CGA	GTA	GTG	TCC	CGC	CGC	ACT	CGG	GCA	GAG	ATG	GGC	GGC	CTC	ACG	894
A	Q	A	A	S	W	T	G	T	V	T	A	R	V	T	Q	R	A	G	P	318
GCT	CAG	GCT	GCC	AGC	TGG	ACT	GGC	ACA	GTG	ACA	GCG	CGC	GTG	ACC	CAG	CGA	GCC	GGG	CCC	954
A	A	P	P	P	E	K	E	Q	P	L	L	P	P	P	P	C	P	A	Q	338
GCC	GCC	CCG	CCG	CCG	GAG	AAG	GAG	CAG	CCA	CTG	CTG	CCT	CCA	CCG	CCC	TGT	CCA	GCG	CAG	1014
P	L	G	R	P	R	S	P	S	P	P	E	K	A	Q	P	P	S	P	P	358
CCG	CTG	GGC	AGG	CCC	CGA	TCC	CCT	TCG	CCC	CCC	GAG	AAG	GCT	CAG	CCG	CCT	TCC	CCG	CCC	1074
T	A	S	A	L	D	Y	P	S	E	N	L	A	F	I	D	E	S	S	D	378
ACG	GCC	TCG	GCC	CTG	GAT	TAT	CCC	AGC	GAG	AAC	CTG	GCC	TTC	ATC	GAC	GAG	TCC	TCG	GAT	1134
T	Q	S	E	R	G	C	P	L	P	R	A	P	R	G	R	R	R	P	N	398
ACG	CAG	AGC	GAG	CGC	GGC	TGC	CCG	CTG	CCC	CGC	GCG	CCG	AGA	GGT	CGC	CGC	CGC	CCA	AAT	1194

FIGURE 1

P	P	R	K	P	V	R	P	R	G	P	G	R	P	R	D	K	G	V	P	418
CCC	CCC	AGG	AAG	CCC	GTG	CGG	CCC	CGC	GGC	CCC	GGG	CGT	CCC	CGA	GAC	AAA	GGC	GTG	CCG	1254
V	*																			420
GTG	TAG																			1260

GGGCAGGATCCCTGGCCGGGCCTCTCAAGGGCTTCGTTTCTGCTCTCCCCGGCATGCCTGGCTTC

FIGURE 1 cont.

Signal Peptide Predictions for 12303

Method	Predict	Score	Mat@
SignalP (eukaryote)	YES		47

Note: amino-terminal 70aa used for signal peptide prediction

FIGURE-2

Transmembrane Segments Predicted by MEMSAT

Start	End	Orient	Score
32	50	ins-->out	5.1
116	137	out-->ins	2.9
144	165	ins-->out	5.4
195	219	out-->ins	6.4
226	242	ins-->out	2.4
260	283	out-->ins	4.5

>12303

MTAPQEPPARPLQAGSGAGPAPGRAMRSTLLALLALVLLYLVS GALVFRALEQPHEQQ
 AQRELGEVREKFLRAHPCVSDQELGLLKEVADALGGGADPETNSTSNSSHS AWDLGSAF
 FFSGTIIITTIGYGNVALRTDAGRLFCIFYALVGIP LFGILLAGVGDRLGSSSLRHGIGHIE
 AIFLKWHVPPPELVRLSAMLFLIGCLLFVLTPTTFVFCYMEDWSKLEAIYFVIVTLTTVG
 FGDYVAGADPRQDSPAYQPLVWFWillGLAYFASVLT TIGNWLRVVSRRTRAEMGGLTAQ
 AASWTGTVTARVTQRAGPAAPPPEKEQPLLPPPPCPAQPLGRPRSPSPPEKAQPPSPPTA
 SALDYPSENLA FIDESSDTQSERGCPLPRAPRGRRRPNPPRKPVPRGPRPRDKGVPV

Transmembrane segments for presumed mature peptide

Start	End	Orient	Score
70	91	out-->ins	2.9
98	119	ins-->out	5.4
149	173	out-->ins	6.4
180	196	ins-->out	2.4
214	237	out-->ins	4.5

>12303_mature

ALVFRALEQPHEQQAQRELGEVREKFLRAHPCVSDQELGLLKEVADALGGGADPETNST
 SNSSHS AWDLGSAFFFSGTIIITTIGYGNVALRTDAGRLFCIFYALVGIP LFGILLAGVGD
 RLGSSSLRHGIGHIEAIFLKWHVPPPELVRLSAMLFLIGCLLFVLTPTTFVFCYMEDWSKL
 EAIYFVIVTLTTVGFGDYVAGADPRQDSPAYQPLVWFWillGLAYFASVLT TIGNWLRVV
 SRRTRAEMGGLTAQAASWTGTVTARVTQRAGPAAPPPEKEQPLLPPPPCPAQPLGRPRSP
 SPPEKAQPPSPPTASALDYPSENLA FIDESSDTQSERGCPLPRAPRGRRRPNPPRKPVPRP
 RGPRPRDKGVPV

FIGURE 3

Protein Family / Domain Matches, HMMer Version 2

Searching for complete domains in PFAM

hmm_pfam ~ search a single seq against HMM database

HMMER 2.1.1 (Dec 1998)

Copyright (C) 1992-1998 Washington University School of Medicine

HMMER is freely distributed under the GNU General Public License (GPL).

HMM file: /prod/ddm/seqanal/PFAM/pfam5.0/Pfam
Sequence file: /prod/ddm/wspace/orfanal/oa-script.23453.seq

Query: 12303

Scores for sequence family classification (score includes all domains):

Model	Description	Score	E-value	N
CNG_membrane	Transmembrane region cyclic Nucleotide G	-130.6	2.6	1
7tm_2	7 transmembrane receptor (Secretin famil	-171.7	7	1

Parsed for domains:

Model	Domain	seq-f	seq-t	hmm-f	hmm-t	score	E-value
CNG_membrane	1/1	27	204	1	259	-130.6	2.6
7tm_2	1/1	25	244	1	268	-171.7	7

Alignments of top-scoring domains:

CNG_membrane: domain 1 of 1, from 27 to 204: score -130.6, E = 2.6

```

*->YlkstwFllDvlStlPfdllyifgsgdegsgslfplLrlnRLLRlr
++st+ ++l++l + lly+++g 1
12303 27 -MRSTT---LLALLALVLLYLVSQA-----L- 48

RvaelfdRlekdtafnyfairlikLvcvtllliHwnACvfdiliyYlisd
v++ +++ ++ a r v +l +H Cv d + li
12303 49 -VFRALQPH-----EQQAQRELGEVREKFLRAHP--CVSDQELGLLIKE 90

YdveaerygfgtdtWlyalnndfeepslwtrgitgGPslkrqYitSlyWS
+ +g + +++ + s w +++ +S
12303 91 -----VADALGGGADPETNSTSNSSHSAWDL-----GSAFFFS 123

itTLTTvGyGdpaPvttrEkiFvifdmLfGvllFAyiiGnvtsivvnms
t+ TT+GyG++a +t++ ++F if+ L+G+ lF+ ++ v++
12303 124 GTIITFIGYGNVALRTDAGRLFCIFYALVGIPFGILLAGVGD----- 166

rtaefrtkmdavkefmrklpkrLqerV..lkyfeytWsnksDegldee
+l L+ +++++ + ++W+
12303 167 -----RLGSSLRHGIGHIEAIFLKWHP-----P 190

evleqLPkkLraeI<-*
e+ + L + L+ I
12303 191 ELVRVLSAMLFLLI 204

```

FIGURE 4

6/9

7tm_2: domain 1 of 1, from 25 to 244: score -171.7, E = 7
 *->allLkviytVGyslSLvaLlIAififllFR.....rLhctR.n
 + ++ ++l L L+ ++f ++ +++++ ++ L R+
 12303 25 RAMRSTTLALALLVLLYLVS GALVFRALEqpheqqaqrELGEVReK 71
 yIHlNLfis.....fiLrallfLigdavlqns vgpeshcsnq.vgCkvv
 ++ + ++s+++ +++++++ +g +++++ +s+++s+++++ +
 12303 72 FLRAHPCVSDqelgLLIKEVADALGGG-ADPET--NSTSNSSHsAWDLGS 118
 avflhYfflaNFfWmLvEGLYLytLL...VvevffserkrlrwYiliGW
 a+f+ + ++ ++ ++V++ + r +Y l+
 12303 119 AFFFSGTIITT-----IgygnVALRTDAGRLFCIFYALV-- 152
 GvPavfvvvwaivrlikpkgygeddgClWlsnedntgfwWiikGPvllai
 G+P+ + ++ + + +g + + + ++ + P l+
 12303 153 GIPLFGILLAGVGD-----RLGSSLRH-GIGH--IEAIFLKWHPPELVR 194
 lvNfilfinilriLvtKlresntgesdqyrlvkstLvLlPLLGitwilfl
 ++ lf++i + +L+ +t ++ +
 12303 195 VLSAMLFLIGC-----LLFVLTPTFVF 217
 fapendarGissvvflylflilnSfQGffVallyCFLNgEV<-*
 + +e+ +s + +y+ + + Gf g+
 12303 218 CYMED---WSKLEAIYFVIVTLTTVGF-----GDY 244

FIGURE 4 cont.

7/9

>105542 p99.2 (1) O88454_MOUSE // TRAAK K+ CHANNEL SUBUNIT CHANNEL
Length = 136

Score = 135 (52.6 bits), Expect = 1.8e-19, Sum P(2) = 1.8e-19
Identities = 27/27 (100%), Positives = 27/27 (100%)

Query: 288 RRTRAEMGGLTAQAASWTGTVTARVTQ 314
RRTRAEMGGLTAQAASWTGTVTARVTQ
Sbjct: 1 RRTRAEMGGLTAQAASWTGTVTARVTQ 27

Score = 118 (46.6 bits), Expect = 1.8e-19, Sum P(2) = 1.8e-19
Identities = 24/24 (100%), Positives = 24/24 (100%)

Query: 359 TASALDYPSENLAFIGESSDTQSE 382
TASALDYPSENLAFIGESSDTQSE
Sbjct: 76 TASALDYPSENLAFIGESSDTQSE 99

A

View Prodom 73512 Boxer Showing match

>73512 p99.2 (1) O88454_MOUSE // TRAAK K+ CHANNEL SUBUNIT IONIC CHANNEL
Length = 60

Score = 175 (66.7 bits), Expect = 1.6e-13, P = 1.6e-13
Identities = 33/55 (60%), Positives = 44/55 (80%)

Query: 50 FRALEQPHEQQAQRELGEVREKFLRAHPCVSDQELGLLIKEVADALGGGADPETN 104
F+ALEQPHEQQAQ+++ R++FLR HPCVS + L IK + +ALGGGA+PET+
Sbjct: 6 FQALEQPHEQQAQKKMDHGRDQFLRDHPCVSQKSLEDFIKLLVEALGGGANPETS 60

B

View Prodom 1641 Boxer Showing match

>1641 p99.2 (31) // CHANNEL PROTEIN IONIC POTASSIUM SUBUNIT K+ PUTATIVE
SUBFAMILY K MEMBER
Length = 128

Score = 156 (60.0 bits), Expect = 2.4e-11, P = 2.4e-11
Identities = 32/75 (42%), Positives = 46/75 (61%)

Query: 216 VFCYMED--WSKLEAIYFVIVTLTTVGFQDYVA-GADPRQDSPAYQPLVFWFILLGLAYF 272
VF Y E+ W+ +A YF ++LTT+GFGDYV D + Y P+V+ +IL+GLA
Sbjct: 54 VFSYYENGWNFFDAFYFSFISLTTIGFGDYVPQNHQDYSPQTYPYLPVFIYILIGLAIT 113
Query: 273 ASVLTITIGNWLRVVS 287
VL +G+WL +S
Sbjct: 114 TVVLDLVGHWLEAIS 128

C

FIGURE 5

8/9

View Prodom 98483

D

>98483 p99.2 (1) O88454_MOUSE // TRAAK K+ CHANNEL SUBUNIT IONIC CHANNEL

Length = 27

Score = 115 (45.5 bits), Expect = 8.8e-07, P = 8.8e-07
Identities = 22/25 (88%), Positives = 23/25 (92%)Query: 175 GIGHIEAIFLKWHVPPPELVRLSAM 199
GIGHIEAIFLKWHVPP LVR LSA+
Sbjct: 1 GIGHIEAIFLKWHVPPGLVRSLSAV 25View Prodom 36

E

>36 p99.2 (544) CIN1(7) CIN5(6) CIK4(6) // CHANNEL IONIC PROTEIN POTASSIUM
SUBUNIT VOLTAGE-GATED TRANSMEMBRANE CALCIUM TRANSPORT ION
Length = 265Score = 115 (45.5 bits), Expect = 9.6e-05, P = 9.6e-05
Identities = 26/58 (44%), Positives = 35/58 (60%)Query: 112 SAWDLGSAFFFSGTIITTIGYGNVA-LRTDAGRLFCIFYALVGIPPLFGILLAGVGDR 168
++W S F ITTIGYGN + T GR+ + YALVGIPPL I+L+ +G R+
Sbjct: 152 TSWCFLSGVLFIALPITTTIGYGNFS>FYTQQGRMETMIYALVGIPPLMLIVLSDIGKRI 209Score = 77 (32.2 bits), Expect = 2.0, P = 0.87
Identities = 20/66 (30%), Positives = 38/66 (57%)Query: 102 ETNSTSNSSH-SAWDLGSAFFFSGTIITTIGYGNVALRTDAGRLFCIFYA-LVGIPPLFGI 159
E NS S SH +++ AF+++ +TT+GYG++ +T AG++F + L G+ +
Sbjct: 108 EINSDEMSHFNSYPF--AFWWAVVTMTTVGYGDMYPKTVAGKIFGTSWCFLSGVLFIAL 165Query: 160 LLAGVG 165
+ +G
Sbjct: 166 PITTTIG 171View Prodom 129403

F

>129403 p99.2 (1) Q21094_CAEEL // K01D12.4 PROTEIN
Length = 234Score = 101 (40.6 bits), Expect = 0.0029, P = 0.0029
Identities = 26/56 (46%), Positives = 33/56 (58%)Query: 99 ADPETNSTSNSSHSAWD-LGSAFFFSGTIITTIGYGNVALRTDAGRLFCIFYALVG 153
+D T+ NS S +D LGS FFS T+I+TIG+G RT GR I Y +VG
Sbjct: 107 SDGATSGLLNS-RSRFDHLGS-LFFSATVISTIGFGTSTPRTHLGRFITIVYGVVG 160

View Prodom 32818

G

>32818 p99.2 (2) Q21529(1) Q94253(1) // CHANNEL M110.2 PROTEIN SIMILAR S
CEREVISIAE OUTWARD-RECTIFIER POTASSIUM TOK1 IONIC
Length = 73

Score = 70 (29.7 bits), Expect = 0.074, P = 0.071
Identities = 17/56 (30%), Positives = 28/56 (50%)

Query: 215 FVFCYMEDWSKLEAIYFVIVTLTTVGFGDYVAGADPRQDSPAYQPLVFWFILLGLA 270
F +C + ++ +YF T+ T+GFGD P D P Y +++ I G+A
Sbjct: 22 FAYCKTSNIPYIDGVYFSTTTIFTIGFGDIT----P--DMPVYHMIIF--IAFGVA 69

View Prodom 128432

H

>128432 p99.2 (1) YWS1_CAEEL // HYPOTHETICAL 25.6 KD PROTEIN B0310.1 IN
CHROMOSOME X TRANSMEMBRANE
Length = 228

Score = 74 (31.1 bits), Expect = 1.1, Sum P(2) = 0.68
Identities = 19/48 (39%), Positives = 27/48 (56%)

Query: 123 SGTIITTIGYGNVALRTDAGRLEFCIFYALVGIPFLGILLAGVGDRGLGS 170
S I TTIG +V T G+L + YAL+G PLF ++ +G + S
Sbjct: 86 SFAISTTIGPLDVDDFTTLGKLAVLYALIGTPLFLTVIGQLGKMVTS 133

Score = 40 (19.1 bits), Expect = 1.1, Sum P(2) = 0.68
Identities = 8/20 (40%), Positives = 13/20 (65%)

Query: 221 EDWSKLEAIYFVIVTLTTVG 240
+D +EAI+ + + TTVG
Sbjct: 163 DDVPFIEAIFSIFLQFTTVG 182

FIGURE 5 cont.

SEQUENCE LISTING

<110> Millennium Pharmaceuticals, Inc.

<120> 12303, A NOVEL HUMAN TWIK MOLECULE AND USES THEREOF

<130> MNI-142PC

<140>

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<150> 60/195,734

<151> 2000-04-07

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Met Thr Thr Ala Pro Gln Glu Pro Pro Ala
1 5 10

cgg ccc ctc cag gcg ggc agt gga gct ggc ccg gcg cct ggg cgc gcc 161
Arg Pro Leu Gln Ala Gly Ser Gly Ala Gly Pro Ala Pro Gly Arg Ala
15 20 25

atg cgc agc acc acg ctc ctg gcc ctg ctg gcg ctg gtc ttg ctt tac 209
Met Arg Ser Thr Thr Leu Leu Ala Leu Leu Ala Leu Val Leu Leu Tyr
30 35 40

ttg gtg tct ggt gcc ctg gtg ttc cgg gcc ctg gag cag ccc cac gag 257
Leu Val Ser Gly Ala Leu Val Phe Arg Ala Leu Glu Gln Pro His Glu
45 50 55

cag cag gcc cag agg gag ctg ggg gag gtc cga gag aag ttc ctg agg 305
Gln Gln Ala Gln Arg Glu Leu Gly Glu Val Arg Glu Lys Phe Leu Arg
60 65 70

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Ala His Pro Cys Val Ser Asp Gln Glu Leu Gly Leu Leu Ile Lys Glu
75 80 85 90

gtg gct gat gcc ctg gga ggg ggt gcg gac cca gaa acc aac tcg acc 401
Val Ala Asp Ala Leu Gly Gly Gly Ala Asp Pro Glu Thr Asn Ser Thr
95 100 105

agc aac agc agc cac tca gcc tgg gac ctg ggc agc gcc ttc ttt ttc 449
Ser Asn Ser Ser His Ser Ala Trp Asp Leu Gly Ser Ala Phe Phe Phe
110 115 120

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Ser Gly Thr Ile Ile Thr Thr Ile Gly Tyr Gly Asn Val Ala Leu Arg	
125 130 135	
aca gat gcc ggg cgc ctc ttc tgc atc ttt tat gcg ctg gtg ggg att	545
Thr Asp Ala Gly Arg Leu Phe Cys Ile Phe Tyr Ala Leu Val Gly Ile	
140 145 150	
ccg ctg ttt ggg atc cta ctg gca ggg gtc ggg gac cgg ctg ggc tcc	593
Pro Leu Phe Gly Ile Leu Leu Ala Gly Val Gly Asp Arg Leu Gly Ser	
155 160 165 170	
tcc ctg cgc cat ggc atc ggt cac att gaa gcc atc ttc ttg aag tgg	641
Ser Leu Arg His Gly Ile Gly His Ile Glu Ala Ile Phe Leu Lys Trp	
175 180 185	
cac gtg cca ccg gag cta gta aga gtg ctg tcg gcg atg ctt ttc ctg	689
His Val Pro Pro Glu Leu Val Arg Val Leu Ser Ala Met Leu Phe Leu	
190 195 200	
ctg atc ggc tgc ctg ctc ttt gtc ctc acg ccc acg ttc gtg ttc tgc	737
Leu Ile Gly Cys Leu Leu Phe Val Leu Thr Pro Thr Phe Val Phe Cys	
205 210 215	
tat atg gag gac tgg agc aag ctg gag gcc atc tac ttt gtc ata gtg	785
Tyr Met Glu Asp Trp Ser Lys Leu Glu Ala Ile Tyr Phe Val Ile Val	
220 225 230	
acg ctt acc acc gtg ggc ttt ggc gac tat gtg gcc ggc gcg gac ccc	833
Thr Leu Thr Thr Val Gly Phe Gly Asp Tyr Val Ala Gly Ala Asp Pro	
235 240 245 250	
agg cag gac tcc ccg gcc tat cag ccg ctg gtg tgg ttc tgg atc ctg	881
Arg Gln Asp Ser Pro Ala Tyr Gln Pro Leu Val Trp Phe Trp Ile Leu	
255 260 265	
ctc ggc ctg gct tac ttc gcc tca gtg ctc acc acc atc ggg aac tgg	929
Leu Gly Leu Ala Tyr Phe Ala Ser Val Leu Thr Thr Ile Gly Asn Trp	
270 275 280	
ctg cga gta gtg tcc cgc cgc act cgg gca gag atg ggc ggc ctc acg	977
Leu Arg Val Val Ser Arg Arg Thr Arg Ala Glu Met Gly Gly Leu Thr	
285 290 295	
gct cag gct gcc agc tgg act ggc aca gtg aca gcg cgc gtg acc cag	1025
Ala Gln Ala Ala Ser Trp Thr Gly Thr Val Thr Ala Arg Val Thr Gln	
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Arg Ala Gly Pro Ala Ala Pro Pro Pro Glu Lys Glu Gln Pro Leu Leu	
315 320 325 330	
cct cca ccg ccc tgt cca gcg cag ccg ctg ggc agg ccc cga tcc cct	1121
Pro Pro Pro Pro Cys Pro Ala Gln Pro Leu Gly Arg Pro Arg Ser Pro	
335 340 345	
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Ser Pro Pro Glu Lys Ala Gln Pro Pro Ser Pro Pro Thr Ala Ser Ala	
350 355 360	

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			20					25					30		
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Val	Phe	Arg	Ala	Leu	Glu	Gln	Pro	His	Glu	Gln	Gln	Ala	Gln	Arg	Glu
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Leu	Gly	Glu	Val	Arg	Glu	Lys	Phe	Leu	Arg	Ala	His	Pro	Cys	Val	Ser
65					70					75					80
Asp	Gln	Glu	Leu	Gly	Leu	Leu	Ile	Lys	Glu	Val	Ala	Asp	Ala	Leu	Gly
				85					90					95	
Gly	Gly	Ala	Asp	Pro	Glu	Thr	Asn	Ser	Thr	Ser	Asn	Ser	Ser	His	Ser
			100					105					110		
Ala	Trp	Asp	Leu	Gly	Ser	Ala	Phe	Phe	Phe	Ser	Gly	Thr	Ile	Ile	Thr
		115					120					125			
Thr	Ile	Gly	Tyr	Gly	Asn	Val	Ala	Leu	Arg	Thr	Asp	Ala	Gly	Arg	Leu
	130				135						140				
Phe	Cys	Ile	Phe	Tyr	Ala	Leu	Val	Gly	Ile	Pro	Leu	Phe	Gly	Ile	Leu
145					150					155					160
Leu	Ala	Gly	Val	Gly	Asp	Arg	Leu	Gly	Ser	Ser	Leu	Arg	His	Gly	Ile
				165					170					175	
Gly	His	Ile	Glu	Ala	Ile	Phe	Leu	Lys	Trp	His	Val	Pro	Pro	Glu	Leu
			180					185					190		

4

Val Arg Val Leu Ser Ala Met Leu Phe Leu Leu Ile Gly Cys Leu Leu
 195 200 205
 Phe Val Leu Thr Pro Thr Phe Val Phe Cys Tyr Met Glu Asp Trp Ser
 210 215 220
 Lys Leu Glu Ala Ile Tyr Phe Val Ile Val Thr Leu Thr Thr Val Gly
 225 230 235 240
 Phe Gly Asp Tyr Val Ala Gly Ala Asp Pro Arg Gln Asp Ser Pro Ala
 245 250 255
 Tyr Gln Pro Leu Val Trp Phe Trp Ile Leu Leu Gly Leu Ala Tyr Phe
 260 265 270
 Ala Ser Val Leu Thr Thr Ile Gly Asn Trp Leu Arg Val Val Ser Arg
 275 280 285
 Arg Thr Arg Ala Glu Met Gly Gly Leu Thr Ala Gln Ala Ala Ser Trp
 290 295 300
 Thr Gly Thr Val Thr Ala Arg Val Thr Gln Arg Ala Gly Pro Ala Ala
 305 310 315 320
 Pro Pro Pro Glu Lys Glu Gln Pro Leu Leu Pro Pro Pro Pro Cys Pro
 325 330 335
 Ala Gln Pro Leu Gly Arg Pro Arg Ser Pro Ser Pro Pro Glu Lys Ala
 340 345 350
 Gln Pro Pro Ser Pro Pro Thr Ala Ser Ala Leu Asp Tyr Pro Ser Glu
 355 360 365
 Asn Leu Ala Phe Ile Asp Glu Ser Ser Asp Thr Gln Ser Glu Arg Gly
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Ser	Gly	Ala	Gly	Pro	Ala	Pro	Gly	Arg	Ala	Met	Arg	Ser	Thr	Thr	Leu	
			20					25					30			
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Leu	Ala	Leu	Leu	Ala	Leu	Val	Leu	Leu	Tyr	Leu	Val	Ser	Gly	Ala	Leu	
		35					40					45				
gtg	ttc	cgg	gcc	ctg	gag	cag	ccc	cac	gag	cag	cag	gcc	cag	agg	gag	192
Val	Phe	Arg	Ala	Leu	Glu	Gln	Pro	His	Glu	Gln	Gln	Ala	Gln	Arg	Glu	
	50					55					60					
ctg	ggg	gag	gtc	cga	gag	aag	ttc	ctg	agg	gcc	cat	ccg	tgt	gtg	agc	240
Leu	Gly	Glu	Val	Arg	Glu	Lys	Phe	Leu	Arg	Ala	His	Pro	Cys	Val	Ser	
65					70				75						80	
gac	cag	gag	ctg	ggc	ctc	ctc	atc	aag	gag	gtg	gct	gat	gcc	ctg	gga	288
Asp	Gln	Glu	Leu	Gly	Leu	Leu	Ile	Lys	Glu	Val	Ala	Asp	Ala	Leu	Gly	
				85					90					95		
ggg	ggt	gcg	gac	cca	gaa	acc	aac	tgc	acc	agc	aac	agc	agc	cac	tca	336
Gly	Gly	Ala	Asp	Pro	Glu	Thr	Asn	Ser	Thr	Ser	Asn	Ser	Ser	His	Ser	
		100					105						110			
gcc	tgg	gac	ctg	ggc	agc	gcc	ttc	ttt	ttc	tca	ggg	acc	atc	atc	acc	384
Ala	Trp	Asp	Leu	Gly	Ser	Ala	Phe	Phe	Phe	Ser	Gly	Thr	Ile	Ile	Thr	
	115						120					125				
acc	atc	ggc	tat	ggc	aat	gtg	gcc	ctg	cgc	aca	gat	gcc	ggg	cgc	ctc	432
Thr	Ile	Gly	Tyr	Gly	Asn	Val	Ala	Leu	Arg	Thr	Asp	Ala	Gly	Arg	Leu	
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Phe	Cys	Ile	Phe	Tyr	Ala	Leu	Val	Gly	Ile	Pro	Leu	Phe	Gly	Ile	Leu	
145					150				155						160	
ctg	gca	ggg	gtc	ggg	gac	cgg	ctg	ggc	tcc	tcc	ctg	cgc	cat	ggc	atc	528
Leu	Ala	Gly	Val	Gly	Asp	Arg	Leu	Gly	Ser	Ser	Leu	Arg	His	Gly	Ile	
			165					170						175		
ggt	cac	att	gaa	gcc	atc	ttc	ttg	aag	tgg	cac	gtg	cca	ccg	gag	cta	576
Gly	His	Ile	Glu	Ala	Ile	Phe	Leu	Lys	Trp	His	Val	Pro	Pro	Glu	Leu	
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gta	aga	gtg	ctg	tgc	gcg	atg	ctt	ttc	ctg	ctg	atc	ggc	tgc	ctg	ctc	624
Val	Arg	Val	Leu	Ser	Ala	Met	Leu	Phe	Leu	Leu	Ile	Gly	Cys	Leu	Leu	
	195					200						205				
ttt	gtc	ctc	acg	ccc	acg	ttc	gtg	ttc	tgc	tat	atg	gag	gac	tgg	agc	672
Phe	Val	Leu	Thr	Pro	Thr	Phe	Val	Phe	Cys	Tyr	Met	Glu	Asp	Trp	Ser	
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Lys	Leu	Glu	Ala	Ile	Tyr	Phe	Val	Ile	Val	Thr	Leu	Thr	Thr	Val	Gly	
225					230				235						240	
ttt	ggc	gac	tat	gtg	gcc	ggc	gcg	gac	ccc	agg	cag	gac	tcc	ccg	gcc	768
Phe	Gly	Asp	Tyr	Val	Ala	Gly	Ala	Asp	Pro	Arg	Gln	Asp	Ser	Pro	Ala	
			245					250						255		
tat	cag	ccg	ctg	gtg	tgg	ttc	tgg	atc	ctg	ctc	ggc	ctg	gct	tac	ttc	816
Tyr	Gln	Pro	Leu	Val	Trp	Phe	Trp	Ile	Leu	Leu	Gly	Leu	Ala	Tyr	Phe	

260	265	270	
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cgc act cgg gca gag atg ggc ggc ctc acg gct cag gct gcc agc tgg Arg Thr Arg Ala Glu Met Gly Gly Leu Thr Ala Gln Ala Ala Ser Trp 290 295 300			912
act ggc aca gtg aca gcg cgc gtg acc cag cga gcc ggg ccc gcc gcc Thr Gly Thr Val Thr Ala Arg Val Thr Gln Arg Ala Gly Pro Ala Ala 305 310 315 320			960
ccg ccg ccg gag aag gag cag cca ctg ctg cct cca ccg ccc tgt cca Pro Pro Pro Glu Lys Glu Gln Pro Leu Leu Pro Pro Pro Pro Cys Pro 325 330 335			1008
gcg cag ccg ctg ggc agg ccc cga tcc cct tcg ccc ccc gag aag gct Ala Gln Pro Leu Gly Arg Pro Arg Ser Pro Ser Pro Pro Glu Lys Ala 340 345 350			1056
cag ccg cct tcc ccg ccc acg gcc tcg gcc ctg gat tat ccc agc gag Gln Pro Pro Ser Pro Pro Thr Ala Ser Ala Leu Asp Tyr Pro Ser Glu 355 360 365			1104
aac ctg gcc ttc atc gac gag tcc tcg gat acg cag agc gag cgc ggc Asn Leu Ala Phe Ile Asp Glu Ser Ser Asp Thr Gln Ser Glu Arg Gly 370 375 380			1152
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gtg ccg gtg Val Pro Val			1257