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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07H 21/04, C07K 14/00, C12N 15/63, 15/85, 15/86

A1

(11) International Publication Number: **WO 00/09534**

(43) International Publication Date: 24 February 2000 (24.02.00)

(21) International Application Number: PCT/US99/18556

(22) International Filing Date: 13 August 1999 (13.08.99)

(30) Priority Data:
60/096,570 14 August 1998 (14.08.98) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL ESK POTASSIUM CHANNEL POLYPEPTIDE AND POLYNUCLEOTIDE COMPOSITIONS

(57) Abstract

The present ivention provides a novel potassium channel subunit (ESK) and polynucleotides which identify and encode ESK. The invention also provides expression vectors and host cells comprising nucleic acid sequence encoding ESK. The invention also provides antibodies of ESK and methods of diagnosing and treating diseases associated with expression of ESK, and screening assays employing the polypeptide, nucleotide, and antibody compositions.

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NOVEL ESK POTASSIUM CHANNEL POLYPEPTIDE AND POLYNUCLEOTIDE COMPOSITIONS

5 Field of the Invention

This invention relates to novel human potassium channel polypeptide and polynucleotide compositions, to the production of these compositions, and to the use of the compositions in the diagnosis, prevention, and treatment of disease states.

10 Background of the Invention

Potassium channels are a heterogeneous group of ion channels that allow selective permeation of potassium ions across the plasma membrane, but differ in details of activation mechanism, voltage range of activity, and kinetic properties. (Hille, B. (1992) Ionic Channels of Excitable Membranes, 2nd Ed. Sinauer, Sunderland, MA; Latorre, R. and Miller, C.
15 (1983) J. Memb. Biol. 7:11-30). They contribute to numerous physiological functions, for example, action potential repolarization, cardiac pacemaking, neuron bursting, muscle contraction, hormone secretion, vascular tone regulation, renal ion reabsorption, learning and memory, and cell growth and differentiation.

A majority of the potassium channel genes isolated and characterized to date encode
20 polypeptides containing six probable transmembrane segments and a single pore-forming P domain (*i.e.,* 1P/6TM subunits). Channels comprising a distinct family of 1P/6TM K+ channels, represented by the *Drosophila* 'ether-a-go-go' (eag) K+ channel, contain a putative cyclic nucleotide binding domain (cNBD) in the carboxyl terminus. Although the eag superfamily is somewhat related to the shaker K+ channel superfamily, the structural
25 differences between the two are quite considerable, with less than 20% identity in the hydrophobic core region. The putative cNBD in eag members shares substantial similarity to the cNBD present in cyclic nucleotide-gated cation channels (Warmke *et al.* (1991), Science 252:1560-1564).

Within the eag superfamily, three channel subfamilies are currently known,
30 represented by eag, erg (eag-related gene), and elk (eag-like K+ channel). In general, two members of the same subfamily from different species share ~65-70% amino acid sequence identity in the region spanning S1 through the cNBD segment. In contrast, two different subfamily members within the same species share only about 40-50% amino acid sequence

identity across the same region (Warmke and Ganetzky (1994) P.N.A.S. 91: 3438-3442).

Channels within the eag superfamily from different animal species can often express channel currents with quite different properties. For example, when expressed in *Xenopus* oocytes, *Drosophila* eag channels are permeable to both K+ and Ca++ ions and are modulated by cAMP, while mouse eag channels are not permeable to Ca++ and are not modulated by cAMP (Bruggemann *et al.* (1993) Nature 365:445-448; Ludwig *et al.* (1994), EMBO J.13:4451-4458; Trudea *et al.*(1995), Science 269: 92-95). The three known mammalian members of the erg subfamily, human erg1 (HERG), rat erg2, and rat erg3, are activated by membrane depolarization, but their inactivation is faster than their activation. Unlike erg1 and erg2, which are active above the firing threshold, erg3 is active below firing threshold, and is thus expected to affect the baseline activity of excitable cells. The erg1 channel is abundantly expressed in the heart and brain, while erg2 and erg3 are absent from the heart, but have a wide expression pattern in the nervous system. All three are expressed in some sympathetic ganglia (Si *et al.,* (1997) J. Neurosci. 17(24):9423-9432).

Mutations of channels within the eag superfamily have found to cause profound deficits in animal physiology. For example, mutations in *Drosophila* eag cause excessive membrane excitability in motor neurons which results in leg shaking behavior in mutant flies. Mutations in *Drosophila* erg can lead to paralysis in affected flies. Mutations in HERG have been linked to a fatal cardiac disease, the long QT syndrome type 2 (LQT2; Curran *et al.* (1995) Cell 80:795-803; Sanguientti *et al.* (1995), Cell 81:299-301).

Potassium channels are associated with a variety of disease states. In some diseases and disorders, abnormal ion channels are believed to be causative factors, while other diseases appear to arise from inappropriate regulation of otherwise normal ion channels. Diseases believed to have a particular association with potassium channels include neurological, cardiovascular, musculoskeletal, and proliferative disorders such as cancers.

The discovery of novel channel polypeptides which represent a new subfamily of the eag K+ channel superfamily, and the polynucleotides which encode them, satisfies a need in the art by providing new compositions which are useful in treatment of various diseases associated with ion channel dysfunction.

Summary of the Invention

The invention is based on the discovery of a human K+ channel, ESK1 (eag-similar K+ channel), which is representative of a new ESK subfamily of the eag K+ channel

superfamily. The invention includes an isolated ESK potassium channel polypeptide comprising an amino acid sequence having at least 60 percent, preferably at least 65 to 70 percent, more preferably at least 80 percent, and most preferably at least 90 percent sequence identity to the region corresponding to residues 212-668 of the polypeptide identified herein as SEQ ID NO:2, said region identified herein as SEQ ID NO:5. The invention also includes (i) fragments of ESK, which are capable of interaction with other proteins, peptides, or chemicals, such interaction which alters the functional properties or cellular/subcellular localization of ESK, and (ii) a pharmaceutical composition containing ESK or an ESK fragment. The invention also includes potassium channels comprising one or more ESK polypeptides.

In a specific embodiment the invention includes an isolated ESK1 polypeptide comprising an amino acid sequence having at least 60 percent sequence identity with SEQ ID NO:2. In other embodiments, the polypeptide comprises a sequence at least 70%, 80%, 90%, or 95% identical to SEQ ID NO:2. In more specific embodiments, the polypeptide has the sequence SEQ ID NO:2 or SEQ ID NO:4.

In another aspect the invention includes an isolated polynucleotide having a sequence which encodes ESK as described above, or a sequence complementary to the ESK coding sequence, and a composition comprising the polynucleotide. The polynucleotide may be mRNA, cRNA, DNA, cDNA, genomic DNA, peptide nucleic acid, as well as an antisense analog thereof. The polynucleotide may encode an ESK polypeptide containing a sequence having at least 60% amino acid sequence identity to SEQ ID NO:5. In another embodiment, the polynucleotide encodes an ESK1 polypeptide having at least 70% amino acid sequence identity to SEQ ID NO:2. The polynucleotide may contain, for example, a coding sequence which hybridizes under high-stringency conditions with the polynucleotide sequence identified as SEQ ID NO:1 or the complement thereof. In specific embodiments, the polynucleotide has the sequence identified as SEQ ID NO:1 or SEQ ID NO:3. The invention also contemplates fragments of the polynucleotide, preferably at least 12, more preferably at least 20 or 30 nucleotides in length.

In another embodiment, the invention includes a nucleic acid molecule of at least 12 nucleotides in length which specifically hybridizes under stringent conditions to a polynucleotide sequence encoding any of the polypeptides described above. The nucleic acid molecule may be mRNA, DNA, cDNA, genomic DNA, peptide nucleic acid, as well as an antisense analog thereof. The invention also includes a polynucleotide sequence comprising

the complement of a polynucleotide sequence encoding any of the polypeptides described above.

Also disclosed is a recombinant expression vector containing a polynucleotide encoding ESK or a fragment described above, and, operably linked to the polynucleotide, regulatory elements effective for expression of the protein in a selected host. Preferred coding sequences are given above. In a related aspect, the invention includes a host cell containing the vector.

The invention further includes a method for producing ESK by recombinant techniques, by culturing recombinant prokaryotic or eukaryotic host cells containing nucleic acid sequence encoding ESK under conditions promoting expression of the protein, and subsequent recovery of the protein from the host cell or the cell culture medium.

In still another aspect, the invention includes an antibody specific against ESK. The antibody has diagnostic and therapeutic applications, particularly in treating neurological and neurodegenerative diseases. Treatment methods which employ antisense or coding sequence polynucleotides for inhibiting or enhancing levels of ESK are also contemplated, as are treatment methods which employ antibodies specific against ESK. The invention also includes methods to alter the expression level of ESK by gene therapy techniques to achieve therapeutic benefits in patients.

Diagnostic methods for detecting levels of ESK in specific tissue samples, and for detecting levels of expression of ESK in tissues, also form part of the invention. In one embodiment, a method of detecting a polynucleotide which encodes ESK in a biological sample, involves the steps of: (a) hybridizing the complement of a polynucleotide which encodes ESK to nucleic acid material of a biological sample, thereby forming a hybridization complex, and (b) detecting the hybridization complex, wherein the presence of the complex correlates with the presence of a polynucleotide encoding ESK in the biological sample. Methods for detecting mutations in the coding region of ESK are also contemplated.

Screening methods which employ ESK for identifying a candidate compound capable of binding to and modulating the activity of ESK also form part of the invention. An exemplary method includes (a) contacting a test compound with ESK, (b) measuring the effect of the test compound on the activity of ESK, and (c) selecting the test compound as a candidate compound if its effect on the activity of ESK is above a selected threshold level. The activity measured may be, for example, establishment or modulation of potassium conductance. In one embodiment, the test compound is a component of a combinatorial

library. In another embodiment, the test compound is an antibody specific against the ESK protein.

The invention also includes, in a related aspect, a compound identified by the screening methods described above, including purified agonists and antagonists. The invention further includes a purified antibody which specifically binds to a polypeptide described above.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

Brief Description of the Figures

Figs. 1A-1E show a nucleic acid coding sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of an ESK polypeptide identified herein as human ESK1 (hESK1);

Brief Description of the Sequences

SEQ ID NO:1 is a nucleic acid sequence encoding hESK1;
SEQ ID NO:2 is the deduced amino acid translation of SEQ ID NO:1;
SEQ ID NO:3 is a nucleic acid sequence encoding a putative hESK1 splice variant;
SEQ ID NO:4 is the deduced amino acid translation of the putative hESK1 splice variant;
SEQ ID NO:5 is the amino acid sequence of residues 212 to 668 of SEQ ID NO:2;
SEQ ID NO:6 is the amino acid sequence of hERG (GenBank PID g487738); and
SEQ ID NO:7 is the sequence of human EST U69184 (GenBank NID g2739408; Accession No. U69184).

Detailed Description of the Invention

I. Definitions

Unless otherwise indicated, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (Second Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM *et al.* (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., for definitions and terms of the art. It is to be understood that this invention is not limited to the particular methodology,

protocols, and reagents described, as these may vary.

The term "polypeptide" as used herein refers to a compound made up of a single chain of amino acid residues linked by peptide bonds. The term "protein" as used herein may be synonymous with the term "polypeptide" or may refer, in addition, to a complex of two or 5 more polypeptides.

A "channel" or "channel protein" as used herein refers to a multisubunit protein comprising two or more P-domain-containing polypeptide subunits, and may be formed of multimers of the same polypeptide (a "homomeric" channel) or of different polypeptides (a "heteromeric" channel). Channel proteins may also contain "accessory subunits" which 10 modulate the activity of the channel.

A "polypeptide belonging to the eag superfamily" is a channel subunit which contains a potential P-domain, six predicted transmembrane domains (S1-S6), a cyclic nucleotide binding domain (cNBD), and has about 40% or greater sequence identity, in the region spanning S1 through the cNBD segment, to a corresponding aligned region of another 15 polypeptide member of the eag K+ channel superfamily, such as eag or human erg1 (hERG).

An "eag-similar K+ polypeptide" or "ESK polypeptide" refers to a polypeptide belonging to the eag superfamily and further containing a sequence having at least 60 percent, preferably at least 65 to 70 percent, more preferably at least 80 percent, and most preferably at least 90 percent sequence identity to a region spanning the S1 through cNBD 20 segment corresponding to residues 212-668 of the polypeptide identified herein as SEQ ID NO:2, said region identified herein as SEQ ID NO:5.

"ESK1" refers to an ESK polypeptide comprising a sequence having at least 70 percent, preferably at least 80 percent, more preferably at least 90 percent, and most preferably at least 95 percent sequence identity to SEQ ID NO:2.

25 As used herein, reference to "ESK" or "ESK1" is meant to include the full-length polypeptide and fragments thereof unless the context indicates otherwise.

The term "ESK channel" refers to a multimeric potassium channel comprising at least one ESK polypeptide.

The term "mature ESK" refers to the ESK polypeptide as it exists in the cell after 30 post-translational processing; for example, after removal of a signal sequence.

The term "modified", when referring to a polypeptide of the invention, means a polypeptide which is modified either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques which are well known in

the art. Among the numerous known modifications which may be present include, but are not limited to, acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation, covalent attachment of a lipid or lipid derivative, methylation, myristlyation, pegylation, prenylation, phosphorylation, ubiqutination, or any similar process.

5 The term "biologically active" refers to an ESK having structural, regulatory or biochemical functions of the naturally occurring ESK including, but not limited to, the ability to support potassium ion conductance when self-associated into a homomeric channel, or when associated with other channel polypeptides into a heteromeric channel. Likewise, "immunologically active" defines the capability of a natural, recombinant or synthetic ESK, 10 or any fragment thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "fragment," when referring to ESK, means a polypeptide which has an amino acid sequence which is the same as part of but not all of the amino acid sequence of ESK, which retains at least one of the functions or activities of ESK, or which is capable of 15 interacting with ESK, other proteins, peptides, or other molecules, to alter a function or activity or the cellular/subcellular localization of an ESK channel. Fragments contemplated include, but are not limited to, an ESK fragment which retains the ability to bind a ligand of an ESK channel, an ESK fragment which blocks the binding of a ligand to an ESK channel, or an ESK fragment which retains immunological activity of ESK. The fragment preferably 20 includes at least 20, more preferably at least 50, contiguous amino acid residues of ESK.

The term "portion", when referring to a polypeptide of the invention, means a polypeptide which has an amino acid sequence which is the same as part of the amino acid sequence of the present invention or a variant thereof, which does not necessarily retain any biological function or activity.

25 A "conservative substitution" refers to the substitution of an amino acid in one class by an amino acid in the same class, where a class is defined by common physicochemical amino acid sidechain properties and high substitution frequencies in homologous proteins found in nature (as determined, e.g., by a standard Dayhoff frequency exchange matrix or BLOSUM matrix). Six general classes of amino acid sidechains, categorized as described 30 above, include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution.

A "non-conservative substitution" refers to the substitution of an amino acid in one class with an amino acid from another class; for example, substitution of an Ala, a class II residue, with a class III residue such as Asp, Asn, Glu, or Gln.

"Optimal alignment" is defined as an alignment giving the highest percent identity score. Such alignment can be performed using a variety of commercially available sequence analysis programs, such as the local alignment program LALIGN using a ktup of 1, default parameters and the default PAM. A preferred alignment is the one performed using the CLUSTAL-W program in MacVector, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM similarity matrix.

"Percent sequence identity", with respect to two amino acid or polynucleotide sequences, refers to the percentage of residues that are identical in the two sequences when the sequences are optimally aligned. Thus, 80% amino acid sequence identity means that 80% of the amino acids in two or more optimally aligned polypeptide sequences are identical. If a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the percent identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the "gap" of the first sequence).

A first polypeptide region is said to "correspond" to a second polypeptide region when the regions are essentially co-extensive when the sequences containing the regions are aligned using a sequence alignment program, as above. Corresponding polypeptide regions typically contain a similar, if not identical, number of residues. It will be understood, however, that corresponding regions may contain insertions or deletions of residues with respect to one another, as well as some differences in their sequences.

"Corresponding" polynucleotide or polypeptide fragments typically contain a similar, if not identical, number of residues. It will be understood, however, that corresponding fragments may contain insertions or deletions of residues with respect to one another, as well as some differences in their sequences.

The term "sequence identity" means nucleic acid or amino acid sequence identity in two or more aligned sequences, aligned as defined above.

"Sequence similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. Thus, 80% protein sequence similarity means that 80% of the amino acid residues in two or more aligned protein sequences are conserved amino acid

residues, *i.e.* are conservative substitutions.

"Hybridization" includes any process by which a strand of a nucleic acid joins with a complementary nucleic acid strand through base-pairing. Thus, strictly speaking, the term refers to the ability of the complement of the target sequence to bind to the test sequence, or
5 vice-versa.

"Hybridization conditions" are based on the melting temperature (Tm) of the nucleic acid binding complex or probe and are typically classified by degree of "stringency" of the conditions under which hybridization is measured. For example, "maximum stringency" typically occurs at about Tm-5°C (5° below the Tm of the probe); "high stringency" at about
10 5-10° below the Tm; "intermediate stringency" at about 10-20° below the Tm of the probe; and "low stringency" at about 20-25° below the Tm. Functionally, maximum stringency conditions may be used to identify nucleic acid sequences having strict identity or near-strict identity with the hybridization probe; while high stringency conditions are used to identify nucleic acid sequences having about 80% or more sequence identity with the probe.

15 An example of "high stringency" conditions includes hybridization at about 65°C in about 5x SSPE and washing at about 65°C in about 0.1x SSPE (where 1x SSPE = 0.15 sodium chloride, 0.010 M sodium phosphate, and 0.001 M disodium EDTA).

The term "gene" as used herein means the segment of DNA involved in producing a polypeptide chain; it may include regions preceding and following the coding region, e.g. 5'
20 untranslated (5' UTR) or "leader" sequences and 3' UTR or "trailer" sequences, as well as intervening sequences (introns) between individual coding segments (exons).

An "isolated polynucleotide having a sequence which encodes ESK" is a polynucleotide which contains the coding sequence of ESK (i) in isolation, (ii) in combination with additional coding sequences, such as fusion protein or signal peptide, in
25 which the ESK coding sequence is the dominant coding sequence, (iii) in combination with non-coding sequences, such as introns and control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host, and/or (iv) in a vector or host environment in which the ESK coding sequence is a heterologous gene.

30 The terms "heterologous DNA" and "heterologous RNA" refer to nucleotides that are not endogenous to the cell or part of the genome in which they are present; generally such nucleotides have been added to the cell, by transfection, microinjection, electroporation, or the like. Such nucleotides generally include at least one coding sequence, but this coding

sequence need not be expressed.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The term "fragment," when referring to an ESK coding sequence, means a polynucleotide which has a nucleic acid sequence which is the same as part of but not all of the nucleic acid sequence of the ESK coding sequence. The fragment preferably includes at least 12 contiguous bases of ESK coding sequence.

The term "expression vector" refers to vectors that have the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

The term "substantially purified" refers to molecules, either polynucleotides or polypeptides, that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

A "variant" polynucleotide sequence may encode a "variant" amino acid sequence which is altered by one or more amino acids from the reference polypeptide sequence. The variant polynucleotide sequence may encode a variant amino acid sequence which contains "conservative" substitutions, wherein the substituted amino acid has structural or chemical properties similar to the amino acid which it replaces. In addition, or alternatively, the variant polynucleotide sequence may encode a variant amino acid sequence which contains "non-conservative" substitutions, wherein the substituted amino acid has dissimilar structural or chemical properties to the amino acid which it replaces. Variant polynucleotides may also encode variant amino acid sequences which contain amino acid insertions or deletions, or both. Furthermore, a variant polynucleotide may encode the same polypeptide as the reference polynucleotide sequence but, due to the degeneracy of the genetic code, has a polynucleotide sequence which is altered by one or more bases from the reference polynucleotide sequence.

An "allelic variant" is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

"Alternative splicing" is a process whereby multiple polypeptide isoforms are generated from a single gene, and involves the splicing together of nonconsecutive exons during the processing of some, but not all, transcripts of the gene. Thus a particular exon may be connected to any one of several alternative exons to form messenger RNAs. The alternatively-spliced mRNAs produce polypeptides ("splice variants") in which some parts are common while other parts are different.

"Splice variants" of ESK, when referred to in the context of an mRNA transcript, are mRNAs produced by alternative splicing of coding regions, i.e., exons, from the ESK gene.

"Splice variants" of ESK, when referred to in the context of the protein itself, are ESK translation products which are encoded by alternatively-spliced ESK mRNA transcripts.

A "mutant" amino acid or polynucleotide sequence is a variant amino acid sequence, or a variant polynucleotide sequence which encodes a variant amino acid sequence, which has significantly altered biological activity from that of the naturally occurring protein.

A "deletion" is defined as a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

An "insertion" or "addition" is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring sequence.

A "substitution" results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

The term "modulate" as used herein refers to the change in activity of the polypeptide of the invention. Modulation may relate to an increase or a decrease in biological activity, binding characteristics, or any other biological, functional, or immunological property of the molecule.

The term "agonist" as used herein, refers to a molecule which, when bound to the channel of the present invention, modulates the activity of the channel by inducing, increasing, or prolonging the duration of the biological activity mediated by the channel. Agonists may themselves be polypeptides, nucleic acids, carbohydrates, lipids, or derivatives thereof, or any other ligand which binds to and modulates the activity of the channel.

The term "antagonist" as used herein, refers to a molecule which, when bound to the

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channel of the present invention, modulates the activity of the channel by blocking, decreasing, or shortening the duration of the biological activity mediated by the channel. Antagonists may themselves be polypeptides, nucleic acids, carbohydrates, lipids, or derivatives thereof, or any other ligand which binds to and modulates the activity of the channel.

The term "humanized antibody" refers to antibody molecule in which one or more amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding activity of the antibody.

"Treating a disease" refers to administering a therapeutic substance effective to reduce the symptoms of the disease and/or lessen the severity of the disease.

II. Polynucleotides Encoding ESK

The invention provides an isolated ESK polypeptide and an isolated polynucleotide encoding the polypeptide. As defined more fully in Section III below, ESK (i) represents a new subfamily of the eag potassium channel superfamily, and (ii) contains an amino acid sequence having at least 60 percent sequence identity to a region identified as SEQ ID NO:5. An exemplary ESK polypeptide, human ESK1 (hESK1), has a sequence identified as SEQ ID NO:2.

As shown in Fig. 1, SEQ ID NO:1 is a 3829 base nucleic acid sequence which contains an open reading frame encoding a 1080 amino acid polypeptide identified herein as an hESK1 polypeptide having the sequence SEQ ID NO:2. A putative hESK1 splice variant, containing a 33 nucleotide insertion between nucleotides 2819 and 2820 of SEQ ID NO:1 resulting in the 3862 base sequence identified herein as SEQ ID NO:3, yields a translation product identical to SEQ ID NO:2 except for an 11 amino acid insertion between amino acids 855 and 856 resulting in the 1091 amino acid polypeptide SEQ ID NO:4.

Polynuceotides encoding hESK1 were discovered in a cDNA library derived from human brain. Coding sequences were identified, cloned and sequenced substantially as described in Example 1. Briefly, a biotin-labeled human probe based on a human EST sequence (GenBank Accession No. U69184; SEQ ID NO:7) was used to capture target cDNA molecules from a brain cDNA library by solution hybridization. After secondary and tertiary screening with radiolabeled oligonucleotide or cDNA probes, positive colonies were cultured, and plasmid cDNA isolated and sequenced, resulting in the construction and identification of a nucleic acid sequence identified as SEQ ID NO:1.

Northern analysis performed as described in Example 2 showed that, among the tissues tested, expression of hESK1 transcript was observed only in brain, predominantly forebrain.

5 A. Polynucleotide compositions

The polynucleotides of the invention include sequences which encode ESK and sequences complementary to the coding sequence, and novel fragments of the polynucleotide. The polynucleotides may be in the form of RNA or in the form of DNA, and include mRNA, cRNA, synthetic RNA and DNA and analogs thereof, cDNA, peptide nucleic acid, and 10 genomic DNA. The polynucleotides may be double-stranded or single-stranded, and if single-stranded may be the coding strand or the non-coding (anti-sense, complementary) strand.

In a general embodiment, the polynucleotide hybridizes under stringent conditions, preferably high-stringency conditions, to the sequence identified as SEQ ID NO:1, SEQ ID 15 NO:3, or the complements thereof. Exemplary hybridization conditions are described in Section IIB below. In another embodiment, the polynucleotide encodes an ESK polypeptide containing a sequence having at least 60% amino acid sequence identity to SEQ ID NO:5. In another embodiment, the polynucleotide encodes an ESK1 polypeptide having at least 70% amino acid sequence identity to SEQ ID NO:2 or SEQ ID NO:4. In other embodiments, the 20 polynucleotide of the invention has at least 70%, preferably at least 80% or 90% sequence identity with the sequence identified as SEQ ID NO:1, SEQ ID NO:3, or the complements thereof. In more specific embodiments, the polynucleotide has the sequence identified as SEQ ID NO:1 or SEQ ID NO:3.

The polynucleotides may include the coding sequence of ESK (i) in isolation, (ii) in 25 combination with additional coding sequences, such as fusion protein or signal peptide, in which the ESK coding sequence is the dominant coding sequence, (iii) in combination with non-coding sequences, such as introns and control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host, and/or (iv) in a vector or host environment in which the ESK coding 30 sequence is a heterologous gene.

The polynucleotide may encode a polypeptide fragment of ESK, for example, an extracellular fragment or an intracellular fragment which has been cleaved from a transmembrane domain of ESK.

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The polynucleotides of the present invention may also have the protein coding sequence fused in-frame to a marker sequence which allows for purification of ESK. The marker sequence may be, for example, a hexahistidine tag to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, the marker
5 sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., *et al.* (1984) Cell 37:767).

Also contemplated are novel uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 20 or 30 bases,
10 corresponding to a region of the coding-sequence polynucleotide or the complement thereof. The polynucleotides may be used as probes, primers, antisense agents, and the like, according to known methods.

B. Preparation of polynucleotides

15 The polynucleotides may be obtained by screening cDNA libraries using oligonucleotide probes which can hybridize to or PCR-amplify polynucleotides which encode the ESK and fragments disclosed above. cDNA libraries prepared from a variety of tissues are commercially available and procedures for screening and isolating cDNA clones are well-known to those of skill in the art. Such techniques are described in, for example, Sambrook
20 *et al.* (1989) Molecular Cloning: A Laboratory Manual (2nd Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM *et al.* (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe and are typically classified by degree of "stringency" of the
25 conditions under which hybridization is measured. For example, "maximum stringency" typically occurs at about Tm-5°C (5° below the Tm of the probe); "high stringency" at about 5-10° below the Tm; "intermediate stringency" at about 10-20° below the Tm of the probe; and "low stringency" at about 20-25° below the Tm. Functionally, maximum stringency conditions may be used to identify nucleic acid sequences having strict identity or near-strict
30 identity with the hybridization probe; while high stringency conditions are used to identify nucleic acid sequences having about 80% or more sequence identity with the probe. An example of high stringency conditions includes hybridization at about 65°C in about 5x SSPE and washing conditions of about 65°C in about 0.1x SSPE (where 1x SSPE = 0.15 sodium

chloride, 0.010 M sodium phosphate, and 0.001 M disodium EDTA).

The polynucleotides may be extended to obtain upstream and downstream sequences such as promoters, regulatory elements, and 5' and 3' untranslated regions (UTRs). Extension of the available transcript sequence may be performed by numerous methods known to those of skill in the art, such as PCR or primer extension (Sambrook *et al.*, supra), or by the RACE method using, for example, the Marathon RACE kit (Clontech, Cat. # K1802-1).

Alternatively, the technique of "restriction-site" PCR (Gobinda *et al.* (1993) PCR Methods Applic. 2:318-22), which uses universal primers to retrieve flanking sequence adjacent a known locus, may be employed. First, genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia T *et al.* (1988) Nucleic Acids Res 16:8186). The primers may be designed using OLIGO(R) 4.06 Primer Analysis Software (1992; National Biosciences Inc, Plymouth, Minn.), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Capture PCR (Lagerstrom M *et al.* (1991) PCR Methods Applic 1:111-19) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. Capture PCR also requires multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into a flanking part of the DNA molecule before PCR.

Another method which may be used to retrieve flanking sequences is that of Parker, JD *et al.* (1991; Nucleic Acids Res 19:3055-60). Additionally, one can use PCR, nested primers and PromoterFinder™ libraries to "walk in" genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions. Preferred libraries for screening for full length cDNAs are ones that have been size-selected to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes. A

randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for extension into the 5' nontranslated regulatory region.

The polynucleotides and oligonucleotides of the invention can also be prepared by solid-phase methods, according to known synthetic methods. Typically, fragments of up to about 100 bases are individually synthesized, then joined to form continuous sequences up to several hundred bases.

C. Applications of polynucleotides

The polynucleotide coding sequences and novel oligonucleotides of the invention have a variety of uses in (1) synthesis of ESK, (2) diagnostics, (3) gene mapping, and (4) therapeutics.

C1. Synthesis of ESK

In accordance with the present invention, polynucleotide sequences which encode ESK, splice variants, fragments of the polypeptide, fusion proteins, or functional equivalents thereof, collectively referred to herein as "ESK", may be used in recombinant DNA molecules that direct the expression of ESK in appropriate host cells. Due to the inherent degeneracy of the genetic code, other nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used to clone and express ESK.

As will be understood by those of skill in the art, it may be advantageous to produce ESK-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray, E. *et al.* (1989) Nuc Acids Res 17:477-508) can be selected, for example, to increase the rate of ESK polypeptide expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

The polynucleotide sequences of the present invention can be engineered in order to alter an ESK coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to produce splice variants, etc.

The present invention also includes recombinant constructs comprising one or more

of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the
5 sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are also described in Sambrook, *et al.*, (*supra*).

The present invention also relates to host cells which are genetically engineered with vectors of the invention, and the production of proteins and polypeptides of the invention by
10 recombinant techniques. Host cells are genetically engineered (i.e., transduced, transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or
15 amplifying the ESK gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art.

The polynucleotides of the present invention may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal,
20 nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host. The appropriate DNA sequence may be inserted into the vector by a variety of
25 procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and related sub-cloning procedures are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate transcription control sequence (promoter) to direct mRNA synthesis. Examples of such
30 promoters include: LTR or SV40 promoter, the *E. coli* lac or trp promoter, the phage lambda PL promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation, and a transcription terminator. The vector may also include

appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

5 The vector containing the appropriate DNA sequence as described above, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. Examples of appropriate expression hosts include: bacterial cells, such as *E. coli*, *Streptomyces*, and *Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* and *Spodoptera* Sf9; mammalian cells 10 such as CHO, COS, BHK, HEK 293 or Bowes melanoma; adenoviruses; plant cells, etc. It is understood that not all cells or cell lines will be capable of producing fully functional ESK; for example, bacterial expression is contemplated for the production of fragments of ESK which may not retain all functions of ESK. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein. The invention is not 15 limited by the host cells employed.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for ESK. For example, when large quantities of ESK or fragments thereof are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not 20 limited to, multifunctional *E. coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the ESK coding sequence may be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of beta-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke & Schuster (1989) J. Biol. Chem 264:5503-5509); pET vectors (Novagen, Madison WI); and the like.

25 In the yeast *Saccharomyces cerevisiae* a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. For reviews, see Ausubel *et al.* (supra) and Grant *et al.* (1987; Methods in Enzymology 153:516-544).

In cases where plant expression vectors are used, the expression of a sequence 30 encoding ESK may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV (Brisson *et al.* (1984) Nature 310:511-514) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu *et al.* (1987) EMBO J 6:307-311). Alternatively, plant promoters such as the small subunit of

RUBISCO (Coruzzi et al (1984) EMBO J 3:1671-1680; Broglie *et al.* (1984) Science 224:838-843); or heat shock promoters (Winter J and Sinibaldi RM (1991) Results. Probl. Cell Differ. 17:85-105) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Hobbs S or Murry LE (1992) in McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York, N.Y., pp 191-196; or Weissbach and Weissbach (1988) Methods for Plant Molecular Biology, Academic Press, New York, N.Y., pp 421-463.

ESK may also be expressed in an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* Sf9 cells or in *Trichoplusia* larvae. The ESK coding sequence is cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of ESK coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which ESK is expressed (Smith *et al.* (1983) J Virol 46:584; Engelhard EK *et al.* (1994) Proc Nat Acad Sci 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, an ESK coding sequence may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing ESK in infected host cells (Logan and Shenk (1984) Proc Natl Acad Sci 81:3655-3659). In addition, transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of an ESK coding sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where ESK coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be

enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf D *et al.* (1994) Results Probl Cell Differ 20:125-62; Bittner *et al.* (1987) Methods in Enzymol 153:516-544).

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., and Battey, I. (1986) Basic Methods in Molecular Biology). Cell-free translation systems can also be employed to produce polypeptides using RNAs derived from the DNA constructs of the present invention. ESK cRNA may be microinjected into cells, such as *Xenopus laevis* oocytes, for production of ESK for electrophysiological measurements or other assays.

A host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, BHK, MDCK, 293, WI38, etc. have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein. For practicing certain aspects of the invention, such as electrophysiological measurements described below, it is appreciated that it may be desirable that the host cell lack endogenous functionally expressed potassium channels having current characteristics similar to those exhibited or modulated by the ESK channel described herein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express ESK may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue

culture techniques appropriate to the cell type.

Host cells transformed with a nucleotide sequence encoding ESK may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, 5 membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding ESK can be designed with signal sequences which direct secretion of ESK through a prokaryotic or eukaryotic cell membrane.

ESK may also be expressed as a recombinant protein with one or more additional 10 polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, Wash.). The inclusion of a 15 protease-cleavable polypeptide linker sequence between the purification domain and ESK is useful to facilitate purification. One such expression vector provides for expression of a fusion protein compromising ESK (*e.g.*, a soluble ESK fragment) fused to a polyhistidine region separated by an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography, as described in 20 Porath *et al.* (1992) Protein Expression and Purification 3:263-281) while the enterokinase cleavage site provides a means for isolating ESK from the fusion protein. pGEX vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to ligand-agarose beads (e.g., 25 glutathione-agarose in the case of GST-fusions) followed by elution in the presence of free ligand.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an additional period. Cells 30 are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods,

which are well know to those skilled in the art.

ESK can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

C2. Diagnostic applications

The polynucleotides of the present invention may be used for a variety of diagnostic purposes. The polynucleotides may be used to detect and quantitate expression of ESK in patient's cells, e.g. biopsied tissues, by detecting the presence of mRNA coding for ESK. This assay typically involves obtaining total mRNA from the tissue and contacting the mRNA with a nucleic acid probe. The probe is a nucleic acid molecule of at least 12 nucleotides, preferably at least 20 or at least 30 nucleotides, capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding ESK under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of ESK. This assay can be used to distinguish between absence, presence, and excess expression of ESK and to monitor levels of ESK expression during therapeutic intervention.

The invention also contemplates the use of the polynucleotides as a diagnostic for diseases resulting from inherited defective ESK genes. These genes can be detected by comparing the sequences of the defective (i.e., mutant) ESK gene with that of a normal one. Association of a mutant ESK gene with abnormal ESK activity (for example, abnormal channel activity) may be verified. In addition, mutant ESK genes can be inserted into a suitable vector for expression in a functional assay system as yet another means to verify or identify mutations. Once mutant genes have been identified, one can then screen populations of interest for carriers of the mutant gene.

The invention also includes a method of determining if a subject is at risk for a disorder associated with, e.g., abnormal ESK channel activity. The method involves detecting at least one of a) aberrant modification or mutation of a polynucleotide sequence encoding ESK, b) misregulation and c) aberrant post-translational modification of ESK. In one embodiment, detecting the genetic lesion includes determining the presence of at least

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one of: a deletion of one or more nucleotides from an ESK gene, an addition of one or more nucleotides to an ESK gene, a substitution of one or more nucleotides of the gene, a gross chromosomal arrangement of the gene, an alteration in the level of mRNA transcript of the gene; the presence of an abnormal splicing pattern of an mRNA transcript of the gene; a non-wild type level of ESK protein expression. In a preferred embodiment, a ESK polynucleotide of the present invention is combined with the nucleic acid of a cell and hybridization of the polynucleotide to the nucleic acid is determined. Failure of the polynucleotide to hybridize or a reduction of hybridization signal are indicative of a mutation in the ESK gene.

Individuals carrying mutations in the gene of the present invention may be detected at the DNA level by a variety of techniques. Nucleic acids used for diagnosis may be obtained from a patient's cells, including but not limited to such as from blood, urine, saliva, placenta, tissue biopsy and autopsy material. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki, et al. (1986) Nature 324:163-166) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid of the present invention can be used to identify and analyze mutations in the gene of the present invention. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype.

Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA of the invention or alternatively, radiolabeled antisense DNA sequences of the invention. Sequence changes at specific locations may also be revealed by nuclease protection assays, such RNase and S1 protection or the chemical cleavage method (e.g. Cotton, et al. (1985) Proc. Natl. Acad. Sci. USA 85:4397-4401), or by differences in melting temperatures. "Molecular beacons" (Kostrikis L.G. et al. (1998) Science 279:1228-1229), hairpin-shaped, single-stranded synthetic oligonucleotides containing probe sequences which are complementary to the nucleic acid of the present invention, may also be used to detect point mutations or other sequence changes as well as monitor expression levels of ESK. Such diagnostics would be particularly useful for, *e.g.*, prenatal testing.

Another method for detecting mutations uses two DNA probes which are designed to hybridize to adjacent regions of a target, with abutting bases, where the region of known or suspected mutation(s) is at or near the abutting bases. The two probes may be joined at the abutting bases, e.g., in the presence of a ligase enzyme, but only if both probes are correctly base paired in the region of probe junction. The presence or absence of mutations is then

detectable by the presence or absence of ligated probe.

Also suitable for detecting mutations in the ESK coding sequence are oligonucleotide array methods based on sequencing by hybridization (SBH), as described, for example, in U.S. Patent No. 5,547,839. In a typical method, the DNA target analyte is hybridized with an
5 array of oligonucleotides formed on a microchip. The sequence of the target can then be "read" from the pattern of target binding to the array

C3. Gene mapping

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular
10 location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with
15 disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 12-30 bp) from the ESK cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, which would complicate the amplification process. These primers are then used for PCR
20 screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide
25 primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include in situ hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

30 Fluorescence in situ hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bases. For a review of this technique, see Verma *et al.* (1988) Human Chromosomes: a Manual of Basic Techniques, Pergamon

Press, New York.

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 the OMIM database (Center for Medical Genetics, Johns Hopkins University, Baltimore, MD and National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD). The OMIM gene map presents the cytogenetic map location of disease genes and other expressed genes. The OMIM database provides information on diseases associated with the chromosomal location. Such associations include the results of linkage analysis mapped to this interval, and the correlation of translocations and other chromosomal aberrations in this area with the advent of polygenic diseases, such as cancer.

C4. Therapeutic applications

Polynucleotides which encode ESK, or complements of the polynucleotides, may also be used for therapeutic purposes. Expression of ESK may be modulated through antisense technology, which controls gene expression through complementary polynucleotides, i.e. antisense DNA or RNA, to the control, 5' or regulatory regions of the gene encoding ESK. For example, the 5' coding portion of the polynucleotide sequence which codes for the protein of the present invention is used to design an antisense oligonucleotide of from about 10 to 40 base pairs in length. Oligonucleotides derived from the transcription start site, e.g. between positions -10 and +10 from the start site, are preferred. An antisense DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (Lee *et al.* (1979) Nucl. Acids Res. 6:3073; Cooney *et al.* (1988) Science 241:456; and Dervan *et al.* (1991) Science 251: 1360), thereby preventing transcription and the production of ESK. An antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into ESK protein (Okano (1991) J. Neurochem. 56:560). The antisense constructs can be delivered to cells by procedures known in the art such that the antisense RNA or DNA may be expressed *in vivo.*

The therapeutic polynucleotides of the invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The polypeptides, and agonist and antagonist compounds which are polypeptides,

may also be employed in accordance with the present invention by expression of such polypeptides in vivo, which is often referred to as "gene therapy." Cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle (Yeh P., *et al.* (1997) FASEB J 11:615-623)

Retroviruses from which the retroviral plasmid vectors mentioned above may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, psi-2, psi-AM, PA12, T19-14X, VT-19-17-H2, psi-CRE, psi-CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller (1990; Human Gene Therapy, Vol. 1, pg. 5-14). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide.

Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

The genes introduced into cells may be placed under the control of inducible
5 promoters, such as the radiation-inducible Egr-1 promoter, (Maceri, H.J., *et al.* (1996) Cancer Res 56(19):4311), to stimulate ESK production or antisense inhibition in response to radiation, *e.g.*, radiation therapy for treating tumors.

III. ESK and ESK Channels

10 The invention is based in part on the structural similarity between ESK and other known subunits of the eag potassium channel superfamily, such as human erg1 (hERG). An exemplary ESK polypeptide sequence SEQ ID NO:2 contains 1080 residues, six potential transmembrane domains spanning approximately residues 212 to 239 (S1), 259 to 277 (S2), 297 to 320 (S3), 329 to 349 (S4), 356 to 378 (S5), and 477 to 501 (S6), and potential N-
15 linked glycosylation sites at residues Asn418, Asn425, Asn433, Asn467, and Asn496. The hESK1 sequence SEQ ID NO:2 also contains a potential pore-forming P-domain spanning approximately residues 449 to 468 (P1) and a putative cyclic nucleotide binding domain (cNBD) spanning about residues 601-668. These structural motifs are characteristic of eag channel subunits.

20 The hESK amino acid sequence SEQ ID NO:2 and the hERG sequence SEQ ID NO:6 were aligned using the CLUSTAL-W pairwise alignment program of MacVector™ software (ver. 6.01; Oxford Molecular Ltd, Oxford, UK) using the default pairwise parameters. The proteins have an overall amino acid sequence identity of about 37%, which increases to about 47% identity (216/457 identical residues) in the region spanning the S1
25 through the cNBD segment, corresponding to residues 212-668 of SEQ ID NO:2, the region identified herein as SEQ ID NO:5. The level of sequence identity in the S1-cNBD region indicates that ESK represents a new subfamily of the eag channel superfamily. In general, two members of the same subfamily from different species share ~65-70% amino acid sequence identity in the region spanning S1 through the cNBD segment. In contrast, two
30 different subfamily members within the same species share only about 40-50% amino acid sequence identity across the same region (Warmke and Ganetzky (1994) P.N.A.S. 91: 3438-3442).

The substantially purified ESK of the invention includes a polypeptide containing an

amino acid sequence having at least 60 percent, preferably at least 65 to 70 percent, more preferably at least 80 percent, and most preferably at least 90 or 95 percent sequence identity to SEQ ID NO:5. In other embodiments, the invention includes a polypeptide having at least 70 percent, at least 80 percent, at least 90 percent, or at least 95 percent sequence identity to SEQ ID NO:2 or SEQ ID NO:4, or having the sequence SEQ ID NO:2 or SEQ ID NO:4. The polypeptide may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide. The polypeptide may be in mature and/or modified form, also as defined above. Also contemplated are fragments derived from ESK, which are capable of interacting with other polypeptides, proteins, or other molecules, such interaction which alters the functional properties or the cellular/subcellular localization of the ESK channel. The invention also includes a substantially purified potassium channel which contain at least one ESK subunit.

The polypeptide sequence variations may include those that are considered conserved and non-conserved substitutions, as defined above. Thus, for example, a polypeptide with a sequence having at least 80% sequence identity with the polypeptide identified as SEQ ID NO:2 (1080 amino acids) contains up to 216 amino acid substitutions when optimally aligned as defined above. In a more specific embodiment, the polypeptide has a sequence substantially identical (97-100% identical) to SEQ ID NO:2 or SEQ ID NO:4. ESK may be (i) a polypeptide in which one or more of the amino acid residues in a sequence listed above are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), or (ii) a polypeptide in which one or more of the amino acid residues includes a substituent group, or (iii) a polypeptide in which the ESK is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol (PEG)), or (iv) a polypeptide in which additional amino acids are fused to ESK, or (v) an isolated fragment of the polypeptide. Such fragments, variants and derivatives are deemed to be within the scope of those skilled in the art from the teachings herein. In particular, splice variants of the polypeptide are also contemplated.

A. Preparation of ESK

Recombinant methods for producing and isolating ESK and fragments are described above.

In addition to recombinant production, fragments and portions of ESK may be produced by direct peptide synthesis using solid-phase techniques (cf Stewart *et al.* (1969)

Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield J (1963) J Am Chem Soc 85:2149-2154). In vitro peptide synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif.) in
5 accordance with the instructions provided by the manufacturer. Portions of ESK may be chemically synthesized separately and combined using chemical methods.

The polypeptide may also be obtained by isolation from natural sources, *e.g.*, by affinity purification using the anti- ESK antibody described in the section below. A fragment or fragments corresponding to extracellular regions and/or the intracellular regions
10 of ESK may be cleaved from the membrane-bound regions using limited proteolysis techniques known to those of skill in the art. The amino acid sequence of a fragment so obtained may be used to design nucleotide coding sequence for recombinant production of the fragment.

15 B. Applications of ESK

The ESK polypeptide of the invention has uses in (1) therapeutic treatment methods and (2) drug screening.

B1. Therapeutic uses and compositions

The ESK polypeptide of the invention is generally useful in treating diseases and
20 disorders associated with ion channel dysfunction, such as, for example, forebrain-related neurological disorders including, but not limited to, dementias such as Alzheimer's disease, depressive and manic-depressive disorders, anxiety, panic, and obsessive-compulsive disorders, eating disorders, attention-deficit and hyperactivity disorders, autism, schizophrenia, epilepsy, and neurodegenerative disorders such as Huntington's and
25 Parkinson's diseases.

ESK may self-associate to form a homomeric ESK channel, or may associate with other potassium channel polypeptides to form a heteromeric ESK channel. While not intending to be bound by theory, a polypeptide fragment of ESK, preferably a soluble fragment which binds an agonist of the ESK channel, may be employed to inhibit activity of
30 the ESK channel by binding an agonist which is necessary for ESK channel activity, in effect competing with ESK channel for agonist. An intracellular fragment of ESK, preferably a soluble fragment, may be used to block the interaction of intracellular effector molecules with an intracellular domain of the ESK channel, thus preventing the cellular response induced by

the interaction of the intracellular effector molecule with the ESK channel.

ESK compositions are tested in appropriate *in vitro* and *in vivo* animal models of disease, to confirm efficacy, tissue metabolism, and to estimate dosages, according to methods well known in the art.

5 ESK compositions may be administered by any of a number of routes and methods designed to provide a consistent and predictable concentration of compound at the target organ or tissue. The polypeptide compositions may be administered alone or in combination with other agents, such as stabilizing compounds, and/or in combination with other pharmaceutical agents such as drugs or hormones.

10 ESK compositions may be administered by a number of routes including, but not limited to oral, intravenous, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means. ESK compositions may also be administered via liposomes. Such administration routes and appropriate formulations are generally known to those of skill in the art.

15 For example, the polypeptide may be given topically to the skin or epithelial linings of body cavities, for infections in such regions. Examples of treatable body cavities include the vagina, the rectum and the urethra. Conveniently, the polypeptide would be formulated into suppository form for administration to these areas.

The polypeptide can be given via intravenous or intraperitoneal injection. Similarly, 20 the polypeptide may be injected to other localized regions of the body. The polypeptide may also be administered via nasal insufflation. Enteral administration is also possible. For such administration, the polypeptide should be formulated into an appropriate capsule or elixir for oral administration, or into a suppository for rectal administration.

The foregoing exemplary administration modes will likely require that the 25 polypeptides be formulated into an appropriate carrier, including ointments, gels, suppositories. Appropriate formulations are well known to persons skilled in the art.

Dosage of the polypeptide will vary, depending upon the potency and therapeutic index of the particular polypeptide selected. These parameters are easily determinable by the skilled practitioner. As an example, if the polypeptide inhibits neuronal cell degradation *in* 30 *vitro* at a given concentration, the practitioner will know that the final desired therapeutic concentration will be this range, calculated on the basis of expected biodistribution. An appropriate target concentration is in the ng/kg to low mg/kg range, e.g., 50 ng/kg to 1 mg/kg body weight, for IV administration.

A therapeutic composition for use in the treatment method can include the polypeptide in a sterile injectable solution, the polypeptide in an oral delivery vehicle, or the polypeptide in a nebulized form, all prepared according to well known methods. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

B2. Screening methods

The present invention also includes an assay for identifying molecules, such as synthetic drugs, antibodies, peptides, or other molecules, which have a modulating effect on the activity of an ESK channel, *e.g.* agonists or antagonists of an ESK channel comprising one or more ESK polypeptides of the present invention. Such an assay comprises the steps of providing a functional ESK channel comprising ESK polypeptides encoded by the polynucleotides of the present invention, contacting the ESK channel with one or more molecules to determine the modulating effect of the molecules on the activity of the channel, and selecting from the molecules a candidate molecule capable of modulating ESK channel activity. Such modulating agents are useful in the treatment of disease conditions associated with activation or reduction of ESK channel activity, including but not limited to those described in section B1 above.

ESK, its ligand-binding, catalytic, or immunogenic fragments, or oligopeptides thereof, can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The protein employed in such a test may be membrane-bound, free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between ESK, or an ESK channel, and the agent being tested may be measured. Compounds which inhibit binding between ESK and its agonists may also be measured.

In one embodiment, the screening system includes recombinantly expressed ESK, and the compounds screened are tested for their ability to block (inhibit) or enhance (activate) the potassium current activity of ESK channel. In a functional screening assay, mammalian cell lines or *Xenopus* oocytes which lack ESK are used to express ESK. ESK may be expressed individually or together with other potassium channel subunit polypeptides. Compounds are screened for their relative effectiveness as channel modulators, e.g., activators or inhibitors, by comparing the relative channel occupancy to the extent of ligand-induced activation or inhibition of potassium ion conductance.

The invention also includes, in a related aspect, an ESK channel modulating agent identified by screening methods employing ESK such as those described above.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the ESK channel is described in detail by Geysen in PCT Application WO 84/03564, published on Sep. 13, 1984. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with ESK (as either a soluble extracellular fragment of ESK, or intact ESK solubilized in detergents or in lipid vesicles), and washed. Bound ESK is then detected by methods well known in the art. Substantially purified ESK can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

Antibodies to ESK, as described in Section IV. below, may also be used in screening assays according to methods well known in the art. For example, a "sandwich" assay may be performed, in which an anti- ESK antibody is affixed to a solid surface such as a microtiter plate and solubilized ESK or ESK channel is added. Such an assay can be used to capture compounds which bind to the ESK channel. Alternatively, such an assay may be used to measure the ability of compounds to interfere with the binding of a ligand, such as an agonist, to the ESK channel.

IV. Anti-ESK antibodies

In still another aspect of the invention, purified ESK is used to produce anti-ESK antibodies which have diagnostic and therapeutic uses related to the activity, distribution, and expression of ESK.

Antibodies to ESK may be generated by methods well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library. Antibodies, *i.e.*, those which block ligand binding, are especially preferred for therapeutic use.

ESK for antibody induction does not require biological activity; however, the polypeptide fragment or oligopeptide must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least ten amino acids, preferably

at least 20 amino acids. Preferably they should mimic a portion of the amino acid sequence of the natural protein and may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of an ESK polypeptide may be fused with another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.
5 Procedures well known in the art can be used for the production of antibodies to ESK.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc may be immunized by injection with ESK or any portion, fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include but are not limited to
10 Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants.

Monoclonal antibodies to ESK may be prepared using any technique which provides
15 for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein (1975; Nature 256:495-497), the human B-cell hybridoma technique (Kosbor *et al.* (1983) Immunol Today 4:72; Cote *et al.* (1983) Proc Natl Acad Sci 80:2026-2030) and the EBV-hybridoma technique (Cole, *et al.* (1984) Mol. Cell Biol. 62:109-120).

20 Techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can also be used (Morrison *et al.* (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger *et al.* (1984) Nature 312:604-608; Takeda *et al.* (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain
25 antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single-chain antibodies specific for ESK.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al.* (1989; Proc Natl Acad Sci 86:3833-3837), and
30 Winter G and Milstein C (1991; Nature 349:293-299).

Antibody fragments which contain specific binding sites for ESK may also be generated. For example, such fragments include, but are not limited to, the F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments

which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD *et al.* (1989) Science 256:1275-1281).

5

A. Diagnostic applications

A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between ESK and 10 its specific antibody and the measurement of complex formation. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on ESK is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox DE *et al.* (1983, J Exp Med 158:1211).

Antibodies which specifically bind ESK are useful for the diagnosis of conditions or 15 diseases characterized by expression of ESK. Alternatively, such antibodies may be used in assays to monitor patients being treated with ESK, its agonists, or its antagonists. Diagnostic assays for ESK protein include methods utilizing the antibody and a label to detect ESK or its fragments in extracts of cells, tissues, or biological fluids such as sera. The proteins and antibodies of the present invention may be used with or without modification. Frequently, the 20 proteins and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known in the art.

A variety of protocols for measuring ESK, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescent activated 25 cell sorting (FACS). As noted above, a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on ESK is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, *et al.* (*supra*). Such protocols provide a basis for diagnosing altered or abnormal levels of ESK expression. Normal or standard values for ESK expression are established by 30 combining cell extracts taken from normal subjects, preferably human, with antibody to ESK under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by various methods, preferably by photometric methods. Then, standard values obtained from normal samples may be compared

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with values obtained from samples from subjects potentially affected by disease. Deviation between standard and subject values establishes the presence of disease state.

The antibody assays are useful to determine the level of ESK present in a particular tissue, e.g., biopsied tumor tissue or neuronal tissue, as an indication of whether ESK is being overexpressed or underexpressed in the tissue, or as an indication of how ESK levels are responding to drug treatment.

B. Therapeutic uses

In conditions associated with ESK, such as neurological disorders including but not limited to those described in Section III.B1 above, therapeutic value may be achieved by administering an antibody specific against ESK, to inhibit, for example, binding of an agonist to the ESK channel, or to block the ion pore.

The antibody employed is preferably a humanized monoclonal antibody, or a human Mab produced by known globulin-gene library methods. The antibody is administered typically as a sterile solution by IV injection, although other parenteral routes may be suitable. Typically, the antibody is administered in an amount between about 1-15 mg/kg body weight of the subject. Treatment is continued, e.g., with dosing every 1-7 days, until a therapeutic improvement is seen.

The following examples illustrate but in no way are intended to limit the present invention.

MATERIALS AND METHODS

Unless otherwise indicated, restriction enzymes and DNA modifying enzymes were obtained from New England Biolabs (Beverly, MA) or Boehringer Mannheim (Indianapolis, IN). The Enhanced Chemo-Luminescence (ECL) system were obtained from Amersham Corp. (Arlington, Heights, IL). Nitrocellulose paper was obtained from Schleicher and Schuell (Keene, NH). "pBLUESCRIPT II SK$^-$" was obtained from Stratagene (La Jolla, CA). Materials for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from Bio-Rad Laboratories (Hercules, CA). Other chemicals were purchased from Sigma (St. Louis, MO) or United States Biochemical (Cleveland, OH).

Example 1

Identification of ESK Nucleic Acid Sequences

To isolate ESK cDNA molecules, the procedure of Shepard & Rae (1997; Nucleic Acids Res. 25(15): 3183-3185) was used with some modifications. Briefly, 10 to 20 ug cDNA from a human brain cDNA library (Edge BioSystems, Gaithersburg, MD) was mixed with 50-80 ng biotinylated oligonucleotide, 50 ng of each clamp oligo, and 1 ul of 1N NaOH in a total volume of 10 ul. The oligonucleotide sequences were derived from human EST sequence U69184 (SEQ ID NO:7). After the mixture was incubated at RT for 15-20 min, 40 ul of neutralization solution (0.12 M Tris, pH 7, 2× SSPE, 0.1% Tween 20) was added, and further incubated at 37 - 42°C. Two to three hours later, to the above reaction mix, 20 ul (200 ug) magnetic beads (Dynabeads) was added, and the mixture was further incubated at the above temperature for 30 min.

To recover captured cDNA molecules, the supernatant was removed and the magnetic beads were washed 5 times with 0.5× SSPE, 0.1% Tween 20. The beads were then further washed with TE once or twice. Finally, the captured cDNA was eluted with 10 ul of 0.5× TE at 70°C for 5 min. The eluted plasmid cDNA was then transform into *E. coli* cells and transformants were plated on one or more 15-cm dishes (a few thousand colonies per dish). Bacterial colonies were lifted onto Hybond N filters (Amersham, Arlington Heights, IL) in duplicate.

Filters were screened using a labeled hybridization probe based on the above EST sequence. The filters were prehybridized without probe in the prehybridization solution (5× SSPE, 5×Denhardt's solution, 0.1% SDS) at 45-50 °C for 1 hour, and then hybridized with probe overnight. The filters were then washed twice for 20 minutes each at room temperature in 2× SSPE, 0.1% SDS, and twice for 20 minutes each at 45-50 °C in 2× SSPE, 0.1% SDS. Signals were detected by a few hours of exposure of the filters to X-ray film.

Positive colonies were subjected to secondary and tertiary screenings. Positive colonies from tertiary screening were cultured. Plasmid DNA was isolated from the positive cultures using a Qiagen miniprep kit (Qiagen, Santa Clarita, CA) and sequenced, resulting in the identification of nucleic acid sequences of a human ESK1 potassium channel subunit polypeptide.

Example 2

Northern Analysis

Multiple tissue Northern blots were purchased from Clontech. High Efficiency Hybridization System (HS-114) was purchased from Molecular Research Center (Cincinnati, Ohio). Briefly, the blot was first soaked in prehybridization solution (1% SDS and 0.1M NaCl) for 30 min at room temperature, and then was incubated in HS-114 solution with 100 μg/ml salmon sperm DNA in the absence of probe for a few hours 68 °C. The cDNA probe was then added and the blot was let to hybridize at 68 °C overnight. The blot was then washed under the following conditions: twice in 2× SSC, 0.05% SDS, at room temperature; and twice in 0.1× SSPE, 0.1% SDS, at 50 °C. After washing, the blot was exposed to X-ray film. Experiments performed as described above showed that, among the tissues tested, expression of ESK1 transcript was observed only in brain, predominantly forebrain.

Example 3

Electrophysiological Measurements

I. Whole Cell Patch Clamp Measurements

Potassium currents can be measured by the patch clamp method in the whole cell configuration (Hamill, et al., 1981). Electrode resistances ranging from 2-6 MΩ are appropriate. Recordings can be made with either an Axopatch 1C or Axopatch 200A amplifier (Axon Instruments, Foster City, CA) interfaced to PCLMP6 software (Axon Instruments) for data acquisition and analysis.

Potassium currents are recorded utilizing an external bath solution consisting of (in mM): 140 sodium chloride, 5 potassium chloride, 10 HEPES, 2 calcium chloride, 1 magnesium chloride, and 12 glucose, adjusted to pH 7.4 with sodium hydroxide and 305 mOsM. The internal pipette solution consists of (in mM): 15 sodium chloride, 125 potassium methanesulphonate, 10 HEPES, 11 EGTA, 1 calcium chloride, 2 magnesium chloride and 59 glucose, adjusted to pH 7.4 with potassium hydroxide and 295 mOsM. For test application, cells are placed in a flow through chamber (0.5-1 ml/min). Currents are elicited by changing the voltage from a holding potential of -90 mV to 0 mV, as a step pulse of 30 msec. duration every 15 sec. Data are sampled at 5 KHz and filtered at 1 KHz. Leak and capacitance currents are subtracted after measuring currents elicited by hyperpolarizing pulses.

Example 4

Anticonvulsant Activity: DBA/2 Mouse Seizure Model

DBA/2 mice (18-21 days old; approx. 7-10 g) are obtained from Jackson Laboratories, Bar Harbor, Maine, and are housed for a minimum of three days to acclimate them to laboratory conditions. On the day of the test, mice are injected i.c.v. into the lateral ventricle with vehicle or test compound (total volume: 5 μl) according to standard methods (Jackson and Scheideler, 1996) 30 minutes prior to exposure to sound stimulus. After injection, the mice are individually housed in observation chambers and are observed over the following 30 min. for evidence of shaking behavior (persistent whole body shakes) or any other abnormal behaviors. The animals are exposed to a high intensity sound stimulus (100-110 dB sinusoidal tone at 14 Hz for 30 s). Mice are observed for the presence of clonic and tonic seizures with full hindlimb extension during the 30 s exposure to the sound.

While the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.

IT IS CLAIMED:

1. A substantially purified ESK polypeptide comprising a sequence having at least 60% sequence identity to SEQ ID NO:5.

2. The polypeptide of claim 1, comprising a sequence having at least 70% sequence identity to SEQ ID NO:2.

3. The polypeptide of claim 1, having the sequence SEQ ID NO:2.

4. The polypeptide of claim 1, comprising a sequence having at least 70% sequence identity to SEQ ID NO:4.

5. The polypeptide of claim 1, having the sequence SEQ ID NO:4.

6. An isolated polynucleotide, comprising
(a) a sequence which encodes the polypeptide of claim 1, or
(b) a sequence complementary to the sequence of (a).

7. An isolated polynucleotide comprising a sequence which hybridizes under high-stringency conditions to a polynucleotide having a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, or the complements thereof.

8. The polynucleotide of claim 7, having the sequence SEQ ID NO:1 or SEQ ID NO:3.

9. A recombinant expression vector, comprising
(a) the polynucleotide of claim 6, and
(b) operably linked to said polynucleotide, regulatory sequences effective for expression of the polynucleotide in a selected host.

10. The vector of claim 9, wherein the polynucleotide comprises a sequence having at least 80% identity to SEQ ID NO:1 or SEQ ID NO:3.

11. The vector of claim 10, wherein the polynucleotide has the sequence SEQ ID NO:1 or SEQ ID NO:3.

5 12. A recombinant eukaryotic cell transfected with the vector of claim 9, and having a functional heterologous ESK expressed by said vector carried on the cell surface.

13. The cell of claim 12, wherein said ESK has a sequence which is at least 70%
10 identical to SEQ ID NO:2 or SEQ ID NO:4.

14. A purified antibody which specifically binds to the polypeptide of claim 1.

15. A method for detecting a polynucleotide which encodes ESK in a biological
15 sample, comprising the steps of:

(a) hybridizing to nucleic acid material of said biological sample a polynucleotide fragment derived from the sequence identified as SEQ ID NO:1, said fragment having a length of at least 12 nucleotides, thereby forming a hybridization complex; and

(b) detecting said hybridization complex;

20 wherein the presence of said hybridization complex correlates with the presence of a polynucleotide encoding ESK in said biological sample.

16. A method of identifying a candidate compound capable of modulating ESK channel activity, comprising

25 (a) contacting a test compound with an ESK channel which comprises a polypeptide subunit containing an amino acid sequence at least 60% identical to SEQ ID NO:5, under conditions in which the activity of said ESK channel can be measured,

(b) measuring the effect of the test compound on the activity of said ESK channel, and

30 (c) selecting the test compound as a candidate compound if its effect on the activity of the ESK channel is above a selected threshold level.

17. A method for detecting ESK in a biological sample, comprising the steps of:

(a) contacting with said biological sample the antibody of claim 14, thereby forming

an antibody-antigen complex; and

(b) detecting said antibody-antigen complex;

wherein the presence of said antibody-antigen complex correlates with the presence of ESK in said biological sample.

```
gcccgggatt tgtgggcggc gagggcgcga ggggccgcgc gccatgctcc gggccccgac    60
ggcgcggacg ccccctcgcg cgccagcgtc cggcgcgacc ccggatcccg gtctgcgcat   120
tgccccccga cggctgcgct agggagcgcg gggcccggcg gggggcggcc gagctgggcg   180
ccctcccccg gcgcggagtc cccgcacccc ggagggatgg ggcgggcagc cgcgggcgcc   240

taagatgccg gcc atg cgg ggc ctc ctg gcg ccg cag aac acc ttc ctg       289
               Met Arg Gly Leu Leu Ala Pro Gln Asn Thr Phe Leu
                1               5                  10

gac acc atc gct acg cgc ttc gac ggc acg cac agt aac ttc gtg ctg      337
Asp Thr Ile Ala Thr Arg Phe Asp Gly Thr His Ser Asn Phe Val Leu
        15                  20                  25

ggc aac gcc cag gtg gcg ggg ctc ttc ccc gtg gtc tac tgc tct gat      385
Gly Asn Ala Gln Val Ala Gly Leu Phe Pro Val Val Tyr Cys Ser Asp
    30                  35                  40

ggc ttc tgt gac ctc acg ggc ttc tcc cgg gct gag gtc atg cag cgg      433
Gly Phe Cys Asp Leu Thr Gly Phe Ser Arg Ala Glu Val Met Gln Arg
45                  50                  55                  60

ggc tgt gcc tgc tcc ttc ctt tat ggg cca gac acc agt gag ctc gtc      481
Gly Cys Ala Cys Ser Phe Leu Tyr Gly Pro Asp Thr Ser Glu Leu Val
                65                  70                  75

cgc caa cag atc cgc aag gcc ctg gac gag cac aag gag ttc aag gct      529
Arg Gln Gln Ile Arg Lys Ala Leu Asp Glu His Lys Glu Phe Lys Ala
            80                  85                  90

gag ctg atc ctg tac cgg aag agc ggg ctc ccg ttc tgg tgt ctc ctg      577
Glu Leu Ile Leu Tyr Arg Lys Ser Gly Leu Pro Phe Trp Cys Leu Leu
        95                  100                 105

gat gtg ata ccc ata aag aat gag aaa ggg gag gtg gct ctc ttc cta      625
Asp Val Ile Pro Ile Lys Asn Glu Lys Gly Glu Val Ala Leu Phe Leu
    110                 115                 120

gtc tct cac aag gac atc agc gaa acc aag aac cga ggg ggc ccc gac      673
Val Ser His Lys Asp Ile Ser Glu Thr Lys Asn Arg Gly Gly Pro Asp
125                 130                 135                 140

aga tgg aag gag aca ggt ggt ggc cgg cgc cga tat ggc cgg gca cga      721
Arg Trp Lys Glu Thr Gly Gly Gly Arg Arg Arg Tyr Gly Arg Ala Arg
                145                 150                 155

tcc aaa ggc ttc aat gcc aac cgg cgg cgg agc cgg gcc gtg ctc tac      769
Ser Lys Gly Phe Asn Ala Asn Arg Arg Arg Ser Arg Ala Val Leu Tyr
            160                 165                 170

cac ctg tcc ggg cac ctg cag aag cag ccc aag ggc aag cac aag ctc      817
His Leu Ser Gly His Leu Gln Lys Gln Pro Lys Gly Lys His Lys Leu
        175                 180                 185

aat aag ggg gtg ttt ggg gag aaa cca aac ttg cct gag tac aaa gta      865
Asn Lys Gly Val Phe Gly Glu Lys Pro Asn Leu Pro Glu Tyr Lys Val
    190                 195                 200
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Fig. 1A

```
gcc gcc atc cgg aag tcg ccc ttc atc ctg ttg cac tgt ggg gca ctg      913
Ala Ala Ile Arg Lys Ser Pro Phe Ile Leu Leu His Cys Gly Ala Leu
205                 210                 215                 220

aga gcc acc tgg gat ggc ttc atc ctg ctc gcc aca ctc tat gtg gct      961
Arg Ala Thr Trp Asp Gly Phe Ile Leu Leu Ala Thr Leu Tyr Val Ala
                225                 230                 235

gtc act gtg ccc tac agc gtg tgt gtg agc aca gca cgg gag ccc agt     1009
Val Thr Val Pro Tyr Ser Val Cys Val Ser Thr Ala Arg Glu Pro Ser
            240                 245                 250

gcc gcc cgc ggc ccg ccc agc gtc tgt gac ctg gcc gtg gag gtc ctc     1057
Ala Ala Arg Gly Pro Pro Ser Val Cys Asp Leu Ala Val Glu Val Leu
        255                 260                 265

ttc atc ctt gac att gtg ctg aat ttc cgt acc aca ttc gtg tcc aag     1105
Phe Ile Leu Asp Ile Val Leu Asn Phe Arg Thr Thr Phe Val Ser Lys
    270                 275                 280

tcg ggc cag gtg gtg ttt gcc cca aag tcc att tgc ctc cac tac gtc     1153
Ser Gly Gln Val Val Phe Ala Pro Lys Ser Ile Cys Leu His Tyr Val
285                 290                 295                 300

acc acc tgg ttc ctg ctg gat gtc atc gca gcg ctg ccc ttt gac ctg     1201
Thr Thr Trp Phe Leu Leu Asp Val Ile Ala Ala Leu Pro Phe Asp Leu
                305                 310                 315

cta cat gcc ttc aag gtc aac gtg tac ttc ggg gcc cat ctg ctg aag     1249
Leu His Ala Phe Lys Val Asn Val Tyr Phe Gly Ala His Leu Leu Lys
            320                 325                 330

acg gtg cgc ctg ctg cgc ctg ctg cgc ctg ctt ccg cgg ctg gac cgg     1297
Thr Val Arg Leu Leu Arg Leu Leu Arg Leu Leu Pro Arg Leu Asp Arg
        335                 340                 345

tac tcg cag tac agc gcc gtg gtg ctg aca ctg ctc atg gcc gtg ttc     1345
Tyr Ser Gln Tyr Ser Ala Val Val Leu Thr Leu Leu Met Ala Val Phe
    350                 355                 360

gcc ctg ctc gcg cac tgg gtc gcc tgc gtc tgg ttt tac att ggc cag     1393
Ala Leu Leu Ala His Trp Val Ala Cys Val Trp Phe Tyr Ile Gly Gln
365                 370                 375                 380

cgg gag atc gag agc agc gaa tcc gag ctg cct gag att ggc tgg ctg     1441
Arg Glu Ile Glu Ser Ser Glu Ser Glu Leu Pro Glu Ile Gly Trp Leu
                385                 390                 395

cag gag ctg gcc cgc cga ctg gag act ccc tac tac ctg gtg ggc cgg     1489
Gln Glu Leu Ala Arg Arg Leu Glu Thr Pro Tyr Tyr Leu Val Gly Arg
            400                 405                 410

agg cca gct gga ggg aac agc tcc ggc cag agt gac aac tgc agc agc     1537
Arg Pro Ala Gly Gly Asn Ser Ser Gly Gln Ser Asp Asn Cys Ser Ser
        415                 420                 425
```

Fig. 1B

```
agc agc gag gcc aac ggg acg ggg ctg gag ctg ctg ggc ggc ccg tcg    1585
Ser Ser Glu Ala Asn Gly Thr Gly Leu Glu Leu Leu Gly Gly Pro Ser
    430                 435                 440

ctg cgc agc gcc tac atc acc tcc ctc tac ttc gca ctc agc agc ctc    1633
Leu Arg Ser Ala Tyr Ile Thr Ser Leu Tyr Phe Ala Leu Ser Ser Leu
445                 450                 455                 460

acc agc gtg ggc ttc ggc aac gtg tcc gcc aac acg gac acc gag aag    1681
Thr Ser Val Gly Phe Gly Asn Val Ser Ala Asn Thr Asp Thr Glu Lys
                465                 470                 475

atc ttc tcc atc tgc acc atg ctc atc ggc gcc ctg atg cac gcg gtg    1729
Ile Phe Ser Ile Cys Thr Met Leu Ile Gly Ala Leu Met His Ala Val
            480                 485                 490

gtg ttt ggg aac gtg acg gcc atc atc cag cgc atg tac gcc cgc cgc    1777
Val Phe Gly Asn Val Thr Ala Ile Ile Gln Arg Met Tyr Ala Arg Arg
        495                 500                 505

ttt ctg tac cac agc cgc acg cgc gac ctg cgc gac tac atc cgc atc    1825
Phe Leu Tyr His Ser Arg Thr Arg Asp Leu Arg Asp Tyr Ile Arg Ile
    510                 515                 520

cac cgt atc ccc aag ccc ctc aag cag cgc atg ctg gag tac ttc cag    1873
His Arg Ile Pro Lys Pro Leu Lys Gln Arg Met Leu Glu Tyr Phe Gln
525                 530                 535                 540

gcc acc tgg gcg gtg aac aat ggc atc gac acc acc gag ctg ctg cag    1921
Ala Thr Trp Ala Val Asn Asn Gly Ile Asp Thr Thr Glu Leu Leu Gln
                545                 550                 555

agc ctc cct gac gag ctg cgc gca gac atc gcc atg cac ctg cac aag    1969
Ser Leu Pro Asp Glu Leu Arg Ala Asp Ile Ala Met His Leu His Lys
            560                 565                 570

gag gtc ctg cag ctg cca ctg ttt gag gcg gcc agc cgc ggc tgc ctg    2017
Glu Val Leu Gln Leu Pro Leu Phe Glu Ala Ala Ser Arg Gly Cys Leu
        575                 580                 585

cgg gca ctg tct ctg gcc ctg cgg ccc gcc ttc tgc acg ccg ggc gag    2065
Arg Ala Leu Ser Leu Ala Leu Arg Pro Ala Phe Cys Thr Pro Gly Glu
    590                 595                 600

tac ctc atc cac caa ggc gat gcc ctg cag gcc ctc tac ttt gtc tgc    2113
Tyr Leu Ile His Gln Gly Asp Ala Leu Gln Ala Leu Tyr Phe Val Cys
605                 610                 615                 620

tct ggc tcc atg gag gtg ctc aag ggt ggc acc gtg ctc gcc atc cta    2161
Ser Gly Ser Met Glu Val Leu Lys Gly Gly Thr Val Leu Ala Ile Leu
                625                 630                 635

ggg aag ggc gac ctg atc ggc tgt gag ctg ccc cgg cgg gag cag gtg    2209
Gly Lys Gly Asp Leu Ile Gly Cys Glu Leu Pro Arg Arg Glu Gln Val
            640                 645                 650
```

Fig. 1C

```
gta aag gcc aat gcc gac gtg aag ggg ctg acg tac tgc gtc ctg cag     2257
Val Lys Ala Asn Ala Asp Val Lys Gly Leu Thr Tyr Cys Val Leu Gln
        655                 660                 665

tgt ctg cag ctg gct ggc ctg cac gac agc ctt gcg ctg tac ccc gag     2305
Cys Leu Gln Leu Ala Gly Leu His Asp Ser Leu Ala Leu Tyr Pro Glu
    670                 675                 680

ttt gcc ccg cgc ttc agt cgt ggc ctc cga ggg gag ctc agc tac aac     2353
Phe Ala Pro Arg Phe Ser Arg Gly Leu Arg Gly Glu Leu Ser Tyr Asn
685                 690                 695                 700

ctg ggt gct ggg gga ggc tct gca gag gtg gac acc agc tcc ctg agc     2401
Leu Gly Ala Gly Gly Gly Ser Ala Glu Val Asp Thr Ser Ser Leu Ser
                705                 710                 715

ggc gac aat acc ctt atg tcc acg ctg gag gag aag gag aca gat ggg     2449
Gly Asp Asn Thr Leu Met Ser Thr Leu Glu Glu Lys Glu Thr Asp Gly
            720                 725                 730

gag cag ggc ccc acg gtc tcc cca gcc cca gct gat gag ccc tcc agc     2497
Glu Gln Gly Pro Thr Val Ser Pro Ala Pro Ala Asp Glu Pro Ser Ser
        735                 740                 745

ccc ctg ctg tcc cct ggc tgc acc tcc tca tcc tca gct gcc aag ctg     2545
Pro Leu Leu Ser Pro Gly Cys Thr Ser Ser Ser Ser Ala Ala Lys Leu
    750                 755                 760

cta tcc cca cgt cga aca gca ccc cgg cct cgt cta ggt ggc aga ggg     2593
Leu Ser Pro Arg Arg Thr Ala Pro Arg Pro Arg Leu Gly Gly Arg Gly
765                 770                 775                 780

agg cca ggc agg gca ggg gct ttg aag gct gag gct ggc ccc tct gct     2641
Arg Pro Gly Arg Ala Gly Ala Leu Lys Ala Glu Ala Gly Pro Ser Ala
                785                 790                 795

ccc cca cgg gcc cta gag ggg cta cgg ctg ccc ccc atg cca tgg aat     2689
Pro Pro Arg Ala Leu Glu Gly Leu Arg Leu Pro Pro Met Pro Trp Asn
            800                 805                 810

gtg ccc cca gat ctg agc ccc agg gta gta gat ggc att gaa gac ggc     2737
Val Pro Pro Asp Leu Ser Pro Arg Val Val Asp Gly Ile Glu Asp Gly
        815                 820                 825

tgt ggc tcg gac cag ccc aag ttc tct ttc cgc gtg ggc cag tct ggc     2785
Cys Gly Ser Asp Gln Pro Lys Phe Ser Phe Arg Val Gly Gln Ser Gly
    830                 835                 840

ccg gaa tgt agc agc agc ccc tcc cct gga cca gag agc ggc ctg ctc     2833
Pro Glu Cys Ser Ser Ser Pro Ser Pro Gly Pro Glu Ser Gly Leu Leu
845                 850                 855                 860

act gtt ccc cat ggg ccc agc gag gca agg aac aca gac aca ctg gac     2881
Thr Val Pro His Gly Pro Ser Glu Ala Arg Asn Thr Asp Thr Leu Asp
                865                 870                 875
```

Fig. 1D

```
aag ctt cgg cag gcg gtg aca gag ctg tca gag cag gtg ctg cag atg     2929
Lys Leu Arg Gln Ala Val Thr Glu Leu Ser Glu Gln Val Leu Gln Met
        880                 885                 890

cgg gaa gga ctg cag tca ctt cgc cag gct gtg cag ctt gtc ctg gcg     2977
Arg Glu Gly Leu Gln Ser Leu Arg Gln Ala Val Gln Leu Val Leu Ala
    895                 900                 905

ccc cac agg gag ggt ccg tgc cct cgg gca tcg gga gag ggg ccg tgc     3025
Pro His Arg Glu Gly Pro Cys Pro Arg Ala Ser Gly Glu Gly Pro Cys
    910                 915                 920

cca gcc agc acc tcc ggg ctt ctg cag cct ctg tgt gtg gac act ggg     3073
Pro Ala Ser Thr Ser Gly Leu Leu Gln Pro Leu Cys Val Asp Thr Gly
925                 930                 935                 940

gca tcc tcc tac tgc ctg cag ccc cca gct ggc tct gtc ttg agt ggg     3121
Ala Ser Ser Tyr Cys Leu Gln Pro Pro Ala Gly Ser Val Leu Ser Gly
                945                 950                 955

act tgg ccc cac cct cgt ccg ggg cct cct ccc ctc atg gca ccc tgg     3169
Thr Trp Pro His Pro Arg Pro Gly Pro Pro Pro Leu Met Ala Pro Trp
            960                 965                 970

ccc tgg ggt ccc cca gcg tct cag agc tcc ccc tgg cct cga gcc aca     3217
Pro Trp Gly Pro Pro Ala Ser Gln Ser Ser Pro Trp Pro Arg Ala Thr
        975                 980                 985

gct ttc tgg acc tcc acc tca gac tca gag ccc cct gcc tca gga gac     3265
Ala Phe Trp Thr Ser Thr Ser Asp Ser Glu Pro Pro Ala Ser Gly Asp
    990                 995                 1000

ctc tgc tct gag ccc agc acc cct gcc tcc cct cct cct tct gag gaa     3313
Leu Cys Ser Glu Pro Ser Thr Pro Ala Ser Pro Pro Pro Ser Glu Glu
005                 1010                1015                1020

ggg gct agg act ggg ccc gca gag cct gtg agc cag gct gag gct acc     3361
Gly Ala Arg Thr Gly Pro Ala Glu Pro Val Ser Gln Ala Glu Ala Thr
                1025                1030                1035

agc act gga gag ccc cca cca ggg tca ggg ggc ctg gcc ttg ccc tgg     3409
Ser Thr Gly Glu Pro Pro Pro Gly Ser Gly Gly Leu Ala Leu Pro Trp
            1040                1045                1050

gac ccc cac agc ctg gag atg gtg ctt att ggc tgc cat ggc tct ggc     3457
Asp Pro His Ser Leu Glu Met Val Leu Ile Gly Cys His Gly Ser Gly
        1055                1060                1065

aca gtc cag tgg acc cag gaa gaa ggc aca ggg gtc tgagtaccag          3503
Thr Val Gln Trp Thr Gln Glu Glu Gly Thr Gly Val
    1070                1075                1080

ccctagaact cagcgttgcc aggtgtgctg ccatctgctg ttcggcccaa cctcagagtg   3563
aaggcagggt ggcagcctcc ccacggactc catgcggccc gctggctcag ggcagggagc   3623
ctggaagcaa aggaggacct ggctcctgac tctcagagag gataggctgg atccctgggg   3683
caggcctctc ctcggcctgc tcctctgacc tcccggtctc cctctgcagg ctgggggcag   3743
aggcctgagg acaaggaaga gctttgccat cccctgcatg tgcccctgcc tctacctgtc   3803
cccaaatttt tatattaaaa aaaaaa                                        3829
```

Fig. 1E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/18556

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; C07K 14/00; C12N 15/63, 85, 86
US CL : 536/23.5; 530/350; 435/320.1; 435/363
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 530/350; 435/320.1; 435/363

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,599,673 A (KEATING ET AL) 04 February 1997, col. 25, lines 5-16	1-13,15
A	US 5,602,169 A (HEWAWASAM ET AL) 11 February 1997, col. 14, lines 36-62	16
A	US 5,436,128 A (HARPOLD ET AL) 25 July 1995, col. 31, lines 1-4	16
A	US 5,328,830 A (JANIS ET AL) 12 July 1994, col. 3, lines 22-39	16
A	TRUDEAU et al. HERG, a Human Inward Rectifier in the Voltage-Gated Potassium Channel Family. Science. 07 July 1995, Vol. 269, pages 92-95, especially page 94, Figure 4.	1-13,15-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

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

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 OCTOBER 1999	03 FEB 2000

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/18556

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WARMKE AND GANETZKY. A family of potassium channel genes related to eag in Drosophila and mammals. Proc. Natl. Acad. Sci. USA. April 1994, Vol. 91, pages 3438-3442, especially page 3440, Figure 1.	1-13,15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/18556

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ **Claims Nos.:**
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ **Claims Nos.:** 1-13, 15-16(in part).
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ **Claims Nos.:**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

Claims 1-13, 15-16 are unsearchable to the extent that they require reference to the specified sequences from the sequence listing. Because Applicant has not furnished a machine-readable copy of the sequence listing, no meaningful search of the sequences per se can be carried out by this Authority. However, the subject matter of the claims has been searched to the extent possible with reference to the balance of the description.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-13, 15, drawn to a purified ESK polypeptide sequence, an ESK polynucleotide sequence, an ESK expression vector, and a eukaryotic cell transfected with the ESK expression vector.
Group II, claim 14, drawn to a purified antibody which binds ESK polypeptide.
Group III, claim 16, drawn to a method of identifying a compound that modulates ESK activity.
Group IV, claim 17, drawn to a method for detecting ESK in a biological sample.
The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first recited product, a purified ESK polypeptide sequence, an ESK polynucleotide sequence, an ESK expression vector, and a eukaryotic cell transfected with the ESK expression vector. Further pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.