(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 October 2002 (10.10.2002)

PCT

(10) International Publication Number
WO 2002/079434 A3

(51) International Patent Classification[7]: C12N 15/11, A61K 67/00, C12P 21/00, G01N 33/00

(21) International Application Number: PCT/US2002/009759

(22) International Filing Date: 29 March 2002 (29.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/280,336	29 March 2001 (29.03.2001)	US
60/324,618	24 September 2001 (24.09.2001)	US
Not furnished	28 March 2002 (28.03.2002)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	60/280,336 (CON)
Filed on	29 March 2001 (29.03.2001)
US	60/324,618 (CON)
Filed on	29 September 2001 (29.09.2001)
US	Not furnished (CON)
Filed on	28 March 2002 (28.03.2002)

(71) Applicant *(for all designated States except US)*: **DELTAGEN, INC.** [US/US]; 740 Bay Road, Redwood City, CA 94063 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **ALLEN, Keith, D.** [US/US]; 224 Custer Trail, Cary, NC 27513 (US).

(74) Agents: **BURKE, John, E.** et al.; Deltagen, Inc., 740 Bay Road, Redwood City, CA 94063 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, 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, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 11 March 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TRANSGENIC MICE CONTAINING KIR3.3 POTASSIUM CHANNEL GENE DISRUPTIONS

(57) Abstract: The present invention relates to transgenic animals, as well as compositions and methods relating to the characterization of gene function. Specifically, the present invention provides tansgenic mice comprising mutation sin a Kir3.3 gene. Such transgenic mice are useful as models for disease and for identifying agents that modulate gene expression and gene function, and as potential treatments for various disease states and disease conditions.

WO 2002/079434 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/09759

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/11; A61k 67/00; C12p 21/00; G01N 33/00

US CL : 800/3, 4, 8, 18, 21; 536/23.1; 435/325

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. : 800/3, 4, 8, 18, 21; 536/23.1; 435/325

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)

medline biosis caplus east

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
x	JELACIC et al. Functional and biochemical evidence for G-protein-gated inwardly rectifying K+(GIRK) channels composed of GIRK2 and GIRK3. J. Biol. Chem. 17 November 2000. Vol. 275. No. 46. pg 36211-36216. See GIRK3 knockout mice on pg 36212, col. 2, line 7.	1-11
Y	WICKMAN et al. Abnormal heart rate regulation in GIRK4 knockout mice. Neuron. 1998. Vol. 20. Pg 103-114	1-11
Y	LESAGE et al. mouse GIRK3 sequence. GenBank Accession Number U11860	1-11
A	WICKMAN et al. Structural characteristics of the mouse Girk genes. 2002. Gene. Vol. 284. pg 241-250. See entire article.	1-11
A	MORGAN et al. Opposing mechanisms may regulate cocaine self-administration in G-protein-gated potassium channel subunit knocout mice. Drug and Alcohol Dependence. 1 May 2002. Vol. 66. No Supplement 1. Pg S124. Abstract 464. See entire abstract.	1-11
A	MARKER et al. Hyperalgesia and blunted morphine analgesia in G protein-gated potassium channel subunit knockout mice. NeuroReport. December 2002. Vol. 13. No. 18. pg 2509-2513. see entire article.	1-11

☒ 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 application or patent 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
02 June 2003 (02.06.2003)	14 JAN 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Deborah Reynolds Telephone No. 703-308-1235

Form PCT/ISA/210 (second sheet) (July 1998)

PCT/US02/09759

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TORRECILLA et al. Decreased met-enkephalin-induced current in locus coeruleus neurons from G Protein-gated inwardly rectifying potassium channel knockout mice. Society for Neuroscience abstracts. 2001. Vol. 27. No. 2. pg 2146. see entire article.	1-11
A	SEINO et al. Diverse roles of KATP channels learned from Kir6.2 genetically engineered mice. Diabetes. March 2000. Vol. 49. pg 311-318.	1-11
A	TORRECILLA et al. G-protein-gated potassium channels containing Kir3.2 and Kir3.3 subunits mediate the acute inhibitory effects of opioids on locus ceruleus neurons. J. Neurosci. 1 June 2002. Vol. 22. No. 11. pg 4328-4334. See entire article.	1-11
A	ZINGMAN et al. Kir6.2 is required for adaptation to stress. PNAS. 1 October 2002. Vol. 99. No. 20. Pg 13278-13283. See entire article.	1-11
A	ZARITSKY et al. The consequences of disrupting cardiac inwardly rectifying K+ current (IK1) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. J. Physiology. 2001. Vol. 533. No. 3. Pg 697-710. See entire article.	1-11
A	PICCIOTTO et al. Using knockout and transgenic mice to study neurophysiology and behavior. Physiological Reviews. October 1998. Vol. 78. No. 4. pg 1131-1163. See entire article.	1-11
A	A proposed test battery and constellations of specific behvioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Hormones and Behavior. 1997. Vol. 31. Pg 197-211. See entire article.	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/09759

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

2. ☐ Claim Nos.:
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:

3. ☐ Claim 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 Continuation 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.: 1-11

Remark on Protest

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

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions that 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, claims 1-11, drawn to a transgenic mouse having a disruption in a Kir3.3 channel gene and a method of making such a mouse.

Group II, claim 12 and 27, drawn to a construct encoding two nucleic acid sequences homologous to a Kir3.3 channel gene and a selectable marker and a Kir3.3 gene.

Group III, claims 13-18, drawn to cells transfected with a vector encoding two nucleic acid sequences homologous to a Kir3.3 channel gene and a selectable marker, cells isolated from a mouse having a disruption in a Kir3.3 channel gene, and ES cells having a disruption in a Kir3.3 channel gene.

Group IV, claims 19, 22 and 24, drawn to a method identifying compounds by contacting a Kir3.3 channel with test agents.

Group V, claims 20, 21 and 23, drawn to methods of using animals to test compounds.

Group VI, claim 25, drawn to method of using "preparations" from a mouse having a disruption in a Kir3.3 channel gene to test compounds

Group VII, claims 26 and 27, drawn to an agent that modulates Kir3.3 channel protein.

Group VIII, claims 28, drawn to a Kir3.3 channel.

Group IX, claim 29, drawn to a method of making pharmaceuticals that modulate a Kir3.3 channel.

Group X, claim 30, drawn to data associated with the phenotype of a transgenic mouse in an electronic database. The inventions listed as Groups I-X 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:

Inventions I and II lack unity because the mouse of group I can be used as a model of disease while the construct can be used to transfect cells in vitro. The mouse of group I does not require the construct and the construct of group II does not have to be used to make the mouse of group I. In addition, the construct does not necessarily disrupt a Kir3.3 channel gene because it encodes at least two sequences that are homologous to a Kir3.3 channel gene.

Inventions I and III lack unity because the mouse can be used as a model of disease while the cells can be used to isolate protein in vitro. The mouse does not have to be made using a transfected cell or an ES cell as it may occur in nature. A cell comprising the construct may not disrupt a Kir3.3 channel gene because the construct does not necessarily disrupt a Kir3.3 channel gene.

Inventions I and IV lack unity because the mouse can be used as a model of disease while contacting a Kir3.3 channel with a compound can be used to determine the function of the Kir3.3 channel protein. The protocols and reagents required for transgenic mice and for contacting inhibitors and compounds are materially distinct and separate. The mouse does not require the method and the method does not require the mouse.

Inventions I and V lack unity because the mouse can be used as a model of disease or to isolate cells while the method of identifying agents that modulate a Kir3.3 channel can be performed with a transgenic or non-transgenic mouse or cells in vitro.

Inventions I and VI lack unity because the mouse may be an in vivo model of disease while the method is used to identify agents that treat disease. The method can be performed with protein, cells or mice.

Inventions I and VII lack unity because the mouse can be used as a model of disease while the modulator of a Kir3.3 channel gene can be used to treat a patient. The protocols and reagents for mice and for using a modulator to treat disease are materially distinct and separate. The mouse does not require the modulator and the modulator does not require the mouse.

Inventions I and VIII lack unity because the mouse can be used as a model of disease while a Kir3.3 channel can be used to isolate antibodies. The protocols and reagents for mice and proteins are materially distinct and separate. The mouse does not require the protein and the protein does not require the mouse.

Inventions I and IX lack unity because the mouse can be used as a model of disease while the method of making pharmaceutical for treating disease are used to make drugs. The protocols and reagents for making mice and making medication are materially distinct and separate. The mouse does not require the method of making medication and the method of making medication does not require the mouse.

Inventions I and X lack unity because the mouse can be used as a model of disease while the data can be used for statistical analysis. The protocols and reagents for transgenic mice and data obtained from transgenic mice are materially distinct and separate. The mouse does not require the data and the data does not require the mouse.

Inventions II and III lack unity because the construct can encode a Kir3.3 channel or disrupt a Kir3.3 channel gene, the construct can be used to make cells in vitro or a mouse, while the cells can be used to isolate protein in vitro or to test compounds

that effect a Kir3.3 channel expression. The construct does not have to be used to make a disruption in a Kir3.3 channel gene. The construct can be used as a probe for a Kir3.3 channel gene.

Inventions II and IV lack unity because the construct can be used to make a Kir3.3 channel in a cell while contacting a Kir3.3 channel with a compound is used to determine if the compound is capable of changing a phenotype. The protocols and reagents required for constructs and for contacting a Kir3.3 channel with test agents are materially distinct and separate. The construct does not require the method and the method does not require the construct.

Inventions II and V lack unity because the construct can be used to make Kir3.3 channel protein or disrupt a Kir3.3 channel gene while the method requires a mouse having a disruption in a Kir3.3 channel gene which includes those made using a construct and those that are naturally occurring. The search required for the construct is different than the search for the steps of the method.

Inventions II and VI lack unity because the construct can be used to make a Kir3.3 channel while the method is used to test agents that treat disease. The protocols and reagents for the product and the method are different. The construct does not require the method and the method does not require the construct.

Inventions II and VII lack unity because the construct can be used to make a Kir3.3 channel or to disrupt a Kir3.3 channel gene while modulators of a Kir3.3 channel gene can be used to treat disease. The protocols and reagents for constructs and modulators are materially distinct and separate. The construct does not require the modulators and the modulators do not require the construct.

Inventions II and VIII lack unity because the construct can be used to make a Kir3.3 channel or to disrupt a Kir3.3 channel gene while a Kir3.3 channel can be used to isolate antibodies. The protocols and reagents required for constructs and proteins are materially distinct and separate. The construct does not require the protein and the protein does not require the construct.

Inventions II and IX lack unity because the construct can be used to make a Kir3.3 channel or to disrupt a Kir3.3 channel gene while the method of making a Kir3.3 channel modulators can be used to make drugs. The protocols and reagents for constructs and drugs are materially distinct and separate. The construct does not require making drugs and the method of making drugs does not require the construct.

Inventions II and X lack unity because the construct can be used to make a Kir3.3 channel or to disrupt a Kir3.3 channel gene while the data can be used for statistical analysis. The protocols and reagents for constructs and data are materially distinct and separate. The construct does not require the data and the data does not require the construct.

Inventions III and IV lack unity because the cells can be used to express a Kir3.3 channel and isolate the Kir3.3 channel protein while contacting a Kir3.3 channel with a test agent can be used to determine the function of the Kir3.3 channel protein. The protocols and reagents required for cells and for contacting proteins with test agents are materially distinct and separate. The cells do not require contacting a protein with test agents and the method does not require the cells.

Inventions III and IV, V or VI lack unity because the cells can be used to isolate protein while the methods are for identifying compounds that treat disease. The cells are not required for the method and the method does not require the cells.

Inventions III and VII lack unity because the cells can be used to study the function of a Kir3.3 channel while the modulators can be used to treat disease. The protocols and reagents for cells and modulators are materially distinct and separate. The cells do not require the modulators and the modulators do not require the cells.

Inventions III and VIII lack unity because the cells can be used to make a Kir3.3 channel while a Kir3.3 channel can be used to isolate antibodies. The protocols and reagents for cells and proteins are materially distinct and separate. The cells do not require the protein and the protein does not require the cells.

Inventions III and IX lack unity because the cells can be used to make a Kir3.3 channel while the method of making modulators can be used to make drugs. The protocols and reagents for cells and drugs are materially distinct and separate. The cells do not require the method and the method does not require the cells.

Inventions III and X lack unity because the cells can be used to make a Kir3.3 channel while the data can be used for statistical analysis. The protocols and reagents for cells and data are materially distinct and separate. The cells do not require the data and the data does not require the cells.

Inventions IV-VI lack unity because identifying agents that modulate a Kir3.3 channel can be performed using a Kir3.3 channel protein, cells having a disruption in a Kir3.3 channel or transgenic mice having a disruption in a Kir3.3 channel gene. The protocols and reagents required to use a Kir3.3 channel, cell having a disruption in a Kir3.3 channel gene and mice having a disruption in a Kir3.3 channel gene are materially distinct and separate than those required to use transgenics. The methods are not disclosed as being used together.

Inventions IV-VI and VII lack unity because the modulators (Invention VII) can be identified using different methods (Inventions IV-VI).

Inventions IV-VI and VIII lack unity because the methods are used to identify agents capable of treating disease while a Kir3.3 channel can be used to isolate antibody. The methods of V and VI do not require the protein. The protein does not require the methods.

Inventions IV-VI and IX lack unity because the method of IV-VI is used to identify agents capable of modulating a Kir3.3 channel while the method of IX is used to make a pharmaceutical. The protocols and reagents for the methods of IV-VI and IX are materially distinct and separate. The methods are not disclosed as being used together.

Inventions IV-VI and X lack unity because the method is used to identify agents capable of modulating a Kir3.3 channel while the data can be used for statistical analysis. The protocols and reagents for the method and for the data are materially distinct and separate. The method does not require the data and the data does not require the method.

Inventions VII and VIII lack unity because modulators can be used to treat disease while a Kir3.3 channel can be used to isolate antibodies. The protocols and reagents required for modulators and a Kir3.3 channel are materially distinct and separate. The protein does not require the modulator and the modulator does not require the protein.

Inventions VII and IX lack unity because the modulators can be used to isolate proteins if the modulator is an antibody while the method is used to make modulators. The protocols and reagents for modulators are materially distinct and separate than those required to make modulators as pharmaceutical. The modulators do not have to be used in the method of making a pharmaceutical. Inventions VII and X lack unity because the modulator can be used to treat disease while the data can be used for statistical analysis. The protocols and reagents for the modulators and for the data are materially distinct and separate. The modulators do not require the data and the data does not require the modulators.

Inventions VIII and IX lack unity because the a Kir3.3 channel can be used to isolate antibodies while making a Kir3.3 channel modulators can be used to make medication. The protocols and reagents for using the protein are materially distinct and separate than those required to make modulators. The protein does not require the method and the method does not require the protein.

Inventions VIII and X lack unity because a Kir3.3 channel can be used to isolate antibodies while the data can be used for statistical analysis. The protocols and reagents for a Kir3.3 channel and for the data are materially distinct and separate. The protein does not require the data and the data does not require the protein.

Inventions IX and X lack unity because the method is used to make medication while the data can be used for statistical analysis. The protocols and reagents for the method and for the data are materially distinct and separate. The method does not require the data and the data does not require the method.