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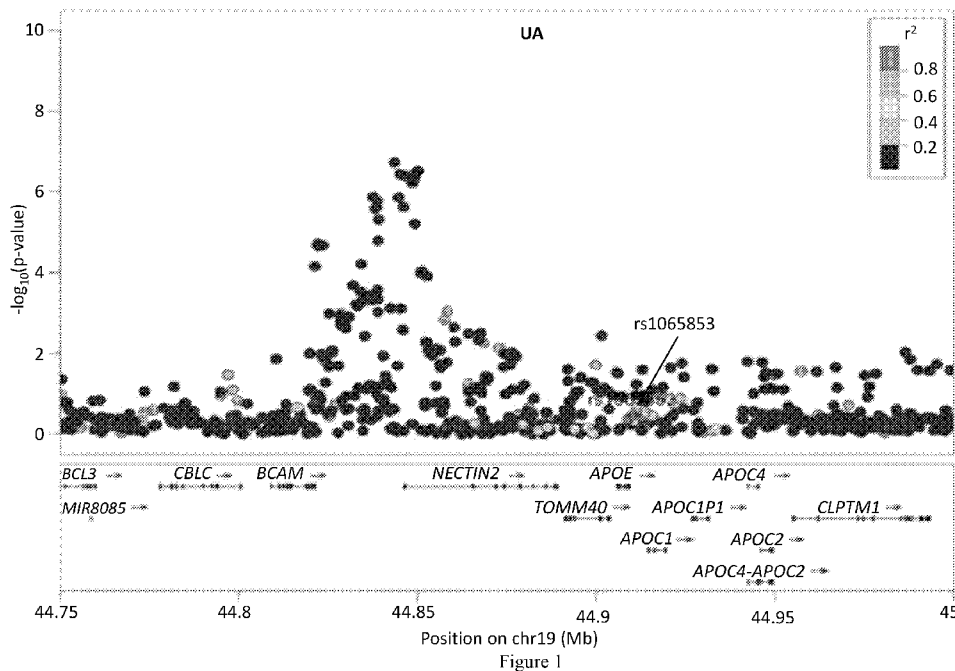
(81) Designated States (unless otherwise indicated, for every kind of national protection available):

AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available):

ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: METHODS OF IMPROVING HEALTH WITH APOLIPOPROTEIN E (APOE) INHIBITORS



(57) Abstract: Methods of increasing longevity and/or inducing healthy aging, and methods of identifying subjects having an increased risk of lower longevity and/or developing one or more age-related diseases are disclosed herein.



WO 2023/141577 A3

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:

24 August 2023 (24.08.2023)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/061008

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/7088 A61P25/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K A61P

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)

EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE, EMBL, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/222065 A1 (ALNYLAM PHARMACEUTICALS INC [US]; BOSTWICK BRET LEE [US] ET AL.) 4 November 2021 (2021-11-04) claims 1,80-110	1-42, 63-73
X	----- WO 2021/061909 A1 (THE J DAVID GLADSTONE INST A TESTAMENTARY TRUST ESTABLISHED UNDER THE) 1 April 2021 (2021-04-01) paragraphs [0004], [0049], [0054]; claims 1-14,21-33,43; example 1	1-42, 63-73
X	----- US 2017/218058 A1 (ROSENTHAL ARNON [US]) 3 August 2017 (2017-08-03) claims 1-7,14,15	1-42, 63-73
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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 but 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

15 May 2023

Date of mailing of the international search report

19/07/2023

Name and mailing address of the ISA/
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Bucka, Alexander

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/061008

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. forming part of the international application as filed.
 - b. furnished subsequent to the international filing date for the purposes of international search (Rule 13^{ter}.1(a)).
 accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/061008

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search 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.:
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. 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 No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional 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 fees, this Authority did not invite payment of additional fees.

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.:
1-42, 63-73

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/061008

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>REA IRENE MAEVE ET AL: "Living long and ageing well: is epigenomics the missing link between nature and nurture?", BIOGERONTOLOGY, SPRINGER NETHERLANDS, DORDRECHT, vol. 17, no. 1, 2 July 2015 (2015-07-02), pages 33-54, XP035901156, ISSN: 1389-5729, DOI: 10.1007/S10522-015-9589-5 [retrieved on 2015-07-02] page 35, right-hand column - page 37, left-hand column</p> <p style="text-align: center;">-----</p>	1-42, 63-73
A	<p>CHIA-CHEN LIU M ET AL: "APOE3-Jacksonville (V236E) variant reduces self-aggregation and risk of dementia", SCI. TRANSL. MED., vol. 13, no. 02, 29 September 2021 (2021-09-29), XP093043487, Retrieved from the Internet: URL:https://www.science.org/doi/pdf/10.1126/SCITRANSLMED.ABC9375> page 1, right-hand column - page 3, left-hand column; table 1</p> <p style="text-align: center;">-----</p>	1-42, 63-73
A	<p>HUYNH TIEN-PHAT V. ET AL: "Age-Dependent Effects of apoE Reduction Using Antisense Oligonucleotides in a Model of [beta]-amyloidosis", NEURON, vol. 96, no. 5, 6 December 2017 (2017-12-06), pages 1013-1023+4, XP055867760, AMSTERDAM, NL ISSN: 0896-6273, DOI: 10.1016/j.neuron.2017.11.014 figures 1,2,4</p> <p style="text-align: center;">-----</p>	1-42, 63-73
A	<p>ANDALEON ANGELA ET AL: "Gene-based association study for lipid traits in diverse cohorts implicates BACE1 and SIDT2 regulation in triglyceride levels", PEERJ, vol. 6, 29 January 2018 (2018-01-29), page e4314, XP093043504, DOI: 10.7717/peerj.4314 Retrieved from the Internet: URL:https://peerj.com/articles/4314.html> page 13 - page 14; figure 1; table 3</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/--</p>	1-42, 63-73

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2023/061008

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SAID M. ABDULLAH ET AL: "Genome-Wide Association Study and Identification of a Protective Missense Variant on Lipoprotein(a) Concentration : Protective Missense Variant on Lipoprotein(a) Concentration-Brief Report", ARTERIOSCLER THROMB VASC BIOL, vol. 41, no. 5, 18 March 2021 (2021-03-18), pages 1792-1800, XP093045141, ISSN: 1079-5642, DOI: 10.1161/ATVBAHA.120.315300 page 1795, right-hand column - page 1796, left-hand column</p> <p>-& Said M Abdullah ET AL: "Supplemental Material Genome wide association study and identification of a protective missense variant on lipoprotein(a) concentration", Arterioscler Thromb Vasc Biol, 18 March 2021 (2021-03-18), XP093045144, Retrieved from the Internet: URL:https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.120.315300/suppl_file/ATVB_ATVB-2020-315300_supp2.pdf [retrieved on 2023-05-09] figures V, VI; tables II, III</p> <p>-----</p>	1-42, 63-73
A	<p>LI ZONGHUA ET AL: "APOE2: protective mechanism and therapeutic implications for Alzheimer's disease", MOLECULAR NEURODEGENERATION, vol. 15, no. 1, 4 November 2020 (2020-11-04), XP093043522, DOI: 10.1186/s13024-020-00413-4 Retrieved from the Internet: URL:http://link.springer.com/article/10.1186/s13024-020-00413-4/fulltext.html> page 5, left-hand column - page 8, left-hand column</p> <p>-----</p>	1-42, 63-73
A	<p>GARATACHEA NURIA ET AL: "ApoE gene and exceptional longevity: Insights from three independent cohorts", EXPERIMENTAL GERONTOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 53, 15 February 2014 (2014-02-15), pages 16-23, XP028841364, ISSN: 0531-5565, DOI: 10.1016/J.EXGER.2014.02.004 page 20, left-hand column - page 22, left-hand column</p> <p>-----</p> <p style="text-align: center;">-/--</p>	1-42, 63-73

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International application No
PCT/US2023/061008

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>MITSURU SHINOHARA ET AL: "APOE2 eases cognitive decline during Aging: Clinical and preclinical evaluations", ANNALS OF NEUROLOGY, JOHN WILEY AND SONS, BOSTON , US, vol. 79, no. 5, 29 March 2016 (2016-03-29) , pages 758-774, XP071641420, ISSN: 0364-5134, DOI: 10.1002/ANA.24628 page 762, right-hand column - page 764, left-hand column; figures 3-5</p> <p>-----</p>	1-42, 63-73
A	<p>ABONDIO PAOLO ET AL: "The Genetic Variability of APOE in Different Human Populations and Its Implications for Longevity", GENES, vol. 10, no. 3, 15 March 2019 (2019-03-15) , page 222, XP093043527, DOI: 10.3390/genes10030222 page 6 - page 8; figure 1</p> <p>-----</p>	1-42, 63-73
A	<p>FERRI E. ET AL: "Apolipoprotein E gene in physiological and pathological aging", MECHANISMS OF AGEING AND DEVELOPMENT., vol. 178, 16 January 2019 (2019-01-16), pages 41-45, XP093043529, CH ISSN: 0047-6374, DOI: 10.1016/j.mad.2019.01.005 Retrieved from the Internet: URL:<https://www.sciencedirect.com/science/article/pii/S0047637418302197/pdf?md5=c0f72c377aecf4f855fddf64ad03e328&pid=1-s2.0-S0047637418302197-main.pdf> table 1</p> <p>-----</p>	1-42, 63-73
A	<p>FENG SHENG ET AL: "ApoE under microscope: Investigating genetic effects on clinical and cognitive outcomes on all genetic variants within the ApoE region using the ADNI next-generation sequencing data", ALZHEIMER'S & DEMENTIA, vol. 11, no. 7, 1 July 2015 (2015-07-01), XP029354459, ISSN: 1552-5260, DOI: 10.1016/J.JALZ.2015.06.1707 abstract</p> <p>-----</p>	1-42, 63-73
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/061008

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LI MENG-YU ET AL: "Investigating Effects of Plasma Apolipoprotein E on Ischemic Heart Disease Using Mendelian Randomization Study", NUTRIENTS, vol. 13, no. 7, 28 June 2021 (2021-06-28), page 2215, XP093045237, DOI: 10.3390/nu13072215 the whole document	1-42, 63-73
A	-& Li Meng-Yu ET AL: "Supplementary Materials - Investigating Effects of Plasma Apolipoprotein E on Ischemic Heart Disease Using Mendelian Randomization Study", Nutrients, 28 June 2021 (2021-06-28), XP093045261, Retrieved from the Internet: URL:https://www.mdpi.com/article/10.3390/nu13072215/s1 [retrieved on 2023-05-09] table S4	1-42, 63-73
X,P	----- US 2022/233569 A1 (FREUDENBERG JAN [US] ET AL) 28 July 2022 (2022-07-28) claims 1,23,43	1-42, 63-73
X,P	----- CN 114 569 720 A (ANIMAL INST OF CHINESE ACADEMY OF SCIENCES) 3 June 2022 (2022-06-03) claims 1-9 -----	1-42, 63-73

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-42, 63-73

A method of increasing longevity in a subject, the method comprising administering an apolipoprotein E (APOE) inhibitor to the subject.

A method of inducing healthy aging in a subject, the method comprising administering an APOE inhibitor to the subject.

2. claims: 43-62

A method of identifying a subject having an increased risk for lower longevity and/or developing one or more age-related diseases, the method comprising: determining or having determined the presence or absence of an apolipoprotein E (APOE) variant nucleic acid molecule in a biological sample obtained from the subject; wherein: when the subject is APOE reference, then the subject has an increased risk for lower longevity and/or developing one or more age-related diseases; and when the subject is heterozygous or homozygous for an APOE variant nucleic acid molecule, then the subject has a decreased risk for lower longevity and/or developing one or more age-related diseases.

3. claims: 74-103

A method of identifying a subject having an increased risk for lower longevity and/or developing one or more age-related diseases, the method comprising: determining or having determined the subject's apolipoprotein E (APOE) score, wherein the APOE score comprises an aggregate of a plurality of APOE variant nucleic acid molecules associated with a decreased risk of lower longevity and/or developing one or more age-related diseases, wherein: an APOE score that is greater than or equal to a threshold APOE score indicates the subject has a decreased risk of lower longevity and/or developing one or more age-related diseases; and an APOE score that is less than a threshold APOE score indicates the subject has an increased risk of lower longevity and/or developing one or more age-related diseases.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2023/061008

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2021222065 A1	04-11-2021	AU 2021263554 A1	08-12-2022
		BR 112022021813 A2	17-01-2023
		CA 3181400 A1	04-11-2021
		CN 115955972 A	11-04-2023
		EP 4143319 A1	08-03-2023
		IL 297702 A	01-12-2022
		JP 2023523993 A	08-06-2023
		KR 20230018377 A	07-02-2023
		US 2023193268 A1	22-06-2023
		WO 2021222065 A1	04-11-2021
<hr style="border-top: 1px dashed black;"/>			
WO 2021061909 A1	01-04-2021	US 2022348638 A1	03-11-2022
		WO 2021061909 A1	01-04-2021
<hr style="border-top: 1px dashed black;"/>			
US 2017218058 A1	03-08-2017	US 2017218058 A1	03-08-2017
		US 2019367588 A1	05-12-2019
		US 2021403540 A1	30-12-2021
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US 2022233569 A1	28-07-2022	NONE	
<hr style="border-top: 1px dashed black;"/>			
CN 114569720 A	03-06-2022	NONE	
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