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(71) Applicants: **INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE)** [FR/FR]; 101, rue de Tolbiac, F-75013 Paris (FR). **UNIVERSITÉ PARIS DESCARTES** [FR/FR]; 12, rue de l'Ecole de Médecine, F-75006 Paris (FR).

(72) Inventors: **POLAK, Michel**; Inserm U845, Faculte De Medecine Paris Descartes, 96 Rue Didot, Batiment Leriche, Cs 61431, F-75993 Paris Cedex 14 (FR). **KARIYAWASAM, Dulanjalee**; Inserm U845, Faculte De Medecine Paris Descartes, 96 Rue Didot, Batiment Leriche, Cs 61431, F-75993 Paris Cedex 14 (FR). **SCHARFMANN, Raphaël**; Inserm U845, Faculte De Medecine Paris Descartes, 96 Rue Didot, Batiment Leriche, CS 61431, F-75993 Paris Cedex 14 (FR). **RACHDI, Latif**; Inserm U845, Faculte De Medecine Paris Descartes, 96 Rue Didot, Batiment Leriche, CS 6143,1, F-75993 Paris Cedex 14 (FR).

(74) Agent: **HIRSCH, Denise**; Inserm Transfert, 7 rue Watt, F-75013 Paris (FR).

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(54) Title: METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF HYPOTHYROIDISM IN DOWN SYNDROME (DS) PATIENTS

(57) Abstract: The present invention relates to a DYRK1A inhibitor or an inhibitor of DYRK1A gene expression for use in the treatment or prevention of hypothyroidism in a Down Syndrome patient.

METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF HYPOTHYROIDISM IN DOWN SYNDROME (DS) PATIENTS

FIELD OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment and prevention of hypothyroidism in Down Syndrome (DS) patients

BACKGROUND OF THE INVENTION:

People with Down syndrome are at an increased risk of developing hypothyroidism. Hypothyroidism is a surprisingly common disorder that can affect people of all ages, and occurs in about 1 in 4,000 people. The incidence is higher in people with Down syndrome. Anywhere from 13 to 55% of people with Down syndrome will develop hypothyroidism over the course of their lifetime. It is unclear why people with Down syndrome have such an increased chance to develop hypothyroidism.

SUMMARY OF THE INVENTION:

The present invention relates to a DYRK1A inhibitor or an inhibitor of DYRK1A gene expression for use in the treatment or prevention of hypothyroidism in a Down Syndrome patient.

DETAILED DESCRIPTION OF THE INVENTION:

The present invention relates to a DYRK1A inhibitor or an inhibitor of DYRK1A gene expression for use in the treatment or prevention of hypothyroidism in a Down Syndrome patient.

In one embodiment, the subject is a foetus diagnosed with Down Syndrome. Down Syndrome diagnosis during pregnancy is generally performed by diagnostic tests such as amniocentesis or chorionic villi sampling (CVS) (the presence of an extra number 21 chromosome on a karyotype).

The term "DYRK1A" has its general meaning in the art and refers to dual-specificity tyrosine phosphorylation-regulated kinase 1A that is a serine/threonine kinase that autophosphorylates on tyrosine residues. The protein contains a nuclear localization signal and has been localized to the splicing-factor compartment (nuclear speckles), but it is also present in the cytoplasm. DYRK1A displays a broad substrate spectrum including transcription factors, splicing factors and synaptic proteins.

The term "DYRKIA inhibitor" refers to a compound able to inhibit phosphorylation induced by DYRKIA. The ability of a compound to inhibit phosphorylation by DYRKIA means that the compound causes a decrease in one or more of the kinase activities evoked by DYRKIA. For example, inhibition of DYRKIA may be shown by compounds exhibiting a Kd of about 5 μM or lower in a DYRKIA binding assay, about 10 μM or lower and about 50 μM or lower.

DYRK1A inhibitors are well known in the art and include for example ,3,4,7-tetrahydroindolo[2,3-c]quinoline compounds as described in US 2011/0136844 2, Imidazaolone derivatives as described in US 2010/0216855, carboxylic acid aryl amides as described in US 2012/0184548, pyrazolo pyrimidines as described in US 2012/0184508, pyrido pyrimidines as described in US 2012/0184542 or naphtthyridines compounds as described in US 2012/0184562. In another embodiment, the DYRK1A inhibitor is Epigallocatechin gallate (EGCG) (Guedj F, Sébrié C, Rivals I, Ledru A, Paly E, Bizot JC, Smith D, Rubin E, Gillet B, Arbones M, Delabar JM. Green tea polyphenols rescue of brain defects induced by overexpression of DYRK1A. PLoS One. 2009;4(2):e4606. Epub 2009 Feb 26.).

Inhibitors of expression for use in the present invention may be based on anti-sense oligonucleotide constructs. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of DYRK1A mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of DYRK1A, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding DYRK1A can be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically inhibiting gene expression of genes

whose sequence is known are well known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

Small inhibitory RNAs (siRNAs) can also function as inhibitors of expression for use in the present invention. DYRK1A gene expression can be reduced by contacting a subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that DYRK1A gene expression is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see Tuschl, T. et al. (1999); Elbashir, S. M. et al. (2001); Hannon, GJ. (2002); McManus, MT. et al. (2002); Brummelkamp, TR. et al. (2002); U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836). All or part of the phosphodiester bonds of the siRNAs of the invention are advantageously protected. This protection is generally implemented via the chemical route using methods that are known by art. The phosphodiester bonds can be protected, for example, by a thiol or amine functional group or by a phenyl group. The 5'- and/or 3'- ends of the siRNAs of the invention are also advantageously protected, for example, using the technique described above for protecting the phosphodiester bonds. The siRNAs sequences advantageously comprises at least twelve contiguous dinucleotides or their derivatives.

As used herein, the term “siRNA derivatives” with respect to the present nucleic acid sequences refers to a nucleic acid having a percentage of identity of at least 90% with erythropoietin or fragment thereof, preferably of at least 95%, as an example of at least 98%, and more preferably of at least 98%.

As used herein, “percentage of identity” between two nucleic acid sequences, means the percentage of identical nucleic acid, between the two sequences to be compared, obtained with the best alignment of said sequences, this percentage being purely statistical and the differences between these two sequences being randomly spread over the nucleic acid acids sequences. As used herein, “ best alignment” or “optimal alignment”, means the alignment for which the determined percentage of identity (see below) is the highest. Sequences comparison between two nucleic acids sequences are usually realized by comparing these sequences that have been previously align according to the best alignment; this comparison is realized on segments of comparison in order to identify and compared the local regions of similarity. The best sequences alignment to perform comparison can be realized, beside by a manual way, by using the global homology algorithm developed by SMITH and WATERMAN (Ad. App. Math., vol.2, p:482, 1981), by using the local homology algorithm developped by

NEDDLEMAN and WUNSCH (J. Mol. Biol., vol.48, p:443, 1970), by using the method of similarities developed by PEARSON and LIPMAN (Proc. Natl. Acd. Sci. USA, vol.85, p:2444, 1988), by using computer softwares using such algorithms (GAP, BESTFIT, BLAST P, BLAST N, FASTA, TFASTA in the Wisconsin Genetics software Package, Genetics Computer Group, 575 Science Dr., Madison, WI USA), by using the MUSCLE multiple alignment algorithms (Edgar, Robert C., Nucleic Acids Research, vol. 32, p:1792, 2004). To get the best local alignment, one can preferably used BLAST software. The identity percentage between two sequences of nucleic acids is determined by comparing these two sequences optimally aligned, the nucleic acids sequences being able to comprise additions or deletions in respect to the reference sequence in order to get the optimal alignment between these two sequences. The percentage of identity is calculated by determining the number of identical position between these two sequences, and dividing this number by the total number of compared positions, and by multiplying the result obtained by 100 to get the percentage of identity between these two sequences.

shRNAs (short hairpin RNA) can also function as inhibitors of expression for use in the present invention.

Ribozymes can also function as inhibitors of expression for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of DYRK1A mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable.

Both antisense oligonucleotides and ribozymes useful as inhibitors of expression can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramadite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that

incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

Antisense oligonucleotides, siRNAs, shRNAs and ribozymes of the invention may be delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the antisense oligonucleotide, siRNA, shRNA or ribozyme nucleic acid to the cells and preferably cells expressing DYRK1A. Preferably, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the antisense oligonucleotide, siRNA, shRNA or ribozyme nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rous sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, 1990 and in Murry, 1991).

Preferred viruses for certain applications are the adenoviruses and adeno-associated (AAV) viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy. Actually 12 different AAV serotypes (AAV1 to 12) are known, each with different tissue tropisms (Wu, Z Mol Ther 2006; 14:316-27). Recombinant AAV are derived from the dependent parvovirus AAV2 (Choi, VW J Virol 2005; 79:6801-07). The adeno-associated virus type 1 to 12 can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species (Wu, Z Mol Ther 2006; 14:316-27). It further has advantages such as, heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g. Sambrook et al., 1989. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are particularly advantageous for this because they do not have the same safety concerns as with many of the viral vectors. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUCl9, pRC/CMV, SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be injected by intramuscular, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation.

In a preferred embodiment, the antisense oligonucleotide, siRNA, shRNA or ribozyme nucleic acid sequence is under the control of a heterologous regulatory region, e.g., a heterologous promoter. The promoter may be specific for Muller glial cells, microglia cells, endothelial cells, pericyte cells and astrocytes For example, a specific expression in Muller glial cells may be obtained through the promoter of the glutamine synthetase gene is suitable. The promoter can also be, e.g., a viral promoter, such as CMV promoter or any synthetic promoters.

The inhibitors of the invention may be administered in the form of a pharmaceutical composition, as defined below. Preferably, said inhibitor in a therapeutically effective amount.

By a "therapeutically effective amount" is meant a sufficient amount of the inhibitor to treat the disease at a reasonable benefit/risk ratio applicable to any medical treatment.

It will be understood that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Preferably, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the inhibitor for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the inhibitor, preferably from 1 mg to about 100 mg of the inhibitor. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In a particular embodiment when the subject is a foetus, the inhibitor is administered to the mother.

The inhibitors of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

The term "Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In the pharmaceutical compositions of the present invention, the inhibitors of the invention can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The inhibitors of the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the inhibitors of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized inhibitors into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the inhibitor plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

In addition to the inhibitors of the invention formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; liposomal formulations; time release capsules; and any other form currently used.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

EXAMPLE: DYRK1A OVEREXPRESSION IS LINKED TO CONGENITAL HYPOTHYROIDISM IN DOWN SYNDROME

Background: Trisomy 21 or Down Syndrome (DS) patients have a predisposition for Congenital Hypothyroidism. This can aggravate their mental status.

The presence of three copies of Dyrk1a gene, localized in chromosome 21 in Humans, may be responsible for a thyroidal dysgenesis in DS patients. Our aim is to understand the molecular mechanisms underlying this hypothesis.

The transgenic Dyrk1a (TgDyrk1a) mouse contains three copies of the Dyrk1a gene. It was obtained through BAC engineering and is widely used as a DS model of study. We have analyzed their thyroid structure and phenotype in whole tissue and in histological sections

using the Image J software at early ages (8-12 weeks old) and assayed their T4 plasmatic levels. In parallel, RNA levels of molecules involved in the thyroidogenesis were studied by qRT-PCR at different embryonic stages and compared between wild type and TgDyrk1a mice.

The average surface of thyroidal follicles in young adult TgDyrk1a mice is larger (TgDyrk1a: 6955μm^2 versus wild type: 5755μm^2; n = 4) but the TgDyrk1a mice presented a lower plasmatic T4 (TgDyrk1a: 2.4 ng/mL versus wild type: 3.7 ng/mL; p = 0.019; n = 14). The overexpression of Dyrk1a in the thyroids leads to a significant elevation of RNA level expression of Nkx2-1(p=0.009), Foxe1 (p=0.025) at E13.5, and Thyroglobulin (p=0.04) at E17.5, all involved in thyroidogenesis. Conclusions: Our first results show an abnormal thyroid function in TgDyrk1a mice. Their thyroidal structure suggests signs of compensation (bigger follicles) presumably linked to TSH increased levels. During thyroidogenesis, factors involved in thyroid development are overexpressed at different stages. To further understand the molecular mechanism linking Dyrk1a overexpression to altered thyroid folliculogenesis and function we are studying candidate genes as direct targets of Dyrk1a using thyroidal cell lines.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

CLAIMS:

1. A method for preventing or treating hypothyroidism in a Down Syndrome patient comprising administering the patient with a therapeutically effective amount of a DYRK1A inhibitor or an inhibitor of DYRK1A gene expression.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/069564

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/353 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)
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, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D KARIYAWASAM ET AL: "Dyrk1A (dual-specificity thyrosine (Y)-phosphorylation regulated kinase 1A) overexpression is linked to congenital hypothyroidism in Down syndrome", 15TH INTERNATIONAL & 14TH EUROPEAN CONGRESS OF ENDOCRINOLOGY (ICE/ECE 2012), vol. 29, May 2012 (2012-05), page P1609, XP055053083, abstract ----- -/--	1

[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 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

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Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2013	31/10/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Rodríguez-Palmero, M

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THIEL ET AL: "Down syndrome and thyroid dysfunction: Should nutritional support be the first-line treatment?", MEDICAL HYPOTHESES, EDEN PRESS, PENRITH, US, vol. 69, no. 4, 2007, pages 809-815, XP022205158, ISSN: 0306-9877, DOI: 10.1016/J.MEHY.2007.01.068 abstract page 881, column 1, last paragraph - column 2, paragraph 1	1
A	KIM D ET AL: "Regulation of Dyrk1A kinase activity by 14-3-3", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 323, no. 2, 3 September 2004 (2004-09-03), pages 499-504, XP027194500, ISSN: 0006-291X, DOI: 10.1016/J.BBRC.2004.08.102 [retrieved on 2004-09-13] abstract	1
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INTERNATIONAL SEARCH REPORT

International application No
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