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(54) **Titre : NOUVELLE CIBLE POUR LE TRAITEMENT ET LA PREVENTION DU DIABETE**
(54) **Title: NEW TARGET FOR DIABETES TREATMENT AND PREVENTION**

(57) **Abrégé/Abstract:**

The present invention relates to the identification of ALMS1 as the missing player involved in the regulation of the insulin-mediated glucose uptake through GLUT4 sorting vesicles, and to the down-regulation of ALMS1 by aPKC. Accordingly, the present invention relates to a molecule capable of preventing the binding of aPKC on ALMS1 for use for treating or preventing diabetes, in particular type 2 diabetes. In addition, the present invention relates to a method for identifying molecule capable of preventing the binding of aPKC on ALMS1.



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(54) Title: NEW TARGET FOR DIABETES TREATMENT AND PREVENTION

(57) Abstract: The present invention relates to the identification of ALMS1 as the missing player involved in the regulation of the insulin-mediated glucose uptake through GLUT4 sorting vesicles, and to the down-regulation of ALMS1 by α PKC. Accordingly, the present invention relates to a molecule capable of preventing the binding of α PKC on ALMS1 for use for treating or preventing diabetes, in particular type 2 diabetes. In addition, the present invention relates to a method for identifying molecule capable of preventing the binding of α PKC on ALMS1.

NEW TARGET FOR DIABETES TREATMENT AND PREVENTION.**FIELD OF THE INVENTION**

The present invention relates to the field of the medicine. More particularly, it relates to diabetes.

5

BACKGROUND OF THE INVENTION

Diabetes mellitus or diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced.

10 There are three main types of diabetes:

- Type 1 results from the body's failure to produce insulin, and currently requires the person to inject insulin or wear an insulin pump.
- Type 2 results from insulin resistance, a condition in which cells fail to use insulin properly.
- 15 - The third one is called gestational diabetes and occurs with pregnant women.

Rates of type 2 diabetes have increased markedly since 1960 in parallel with obesity: As of 2010 there are approximately 285 million people with the disease compared to around 30 million in 1985. Long-term complications from high blood sugar can include heart diseases, strokes, diabetic retinopathy, chronic renal failure which may require dialysis and poor circulation in the
20 limbs leading to amputations. Nonketotic hyperosmolar coma may occur.

It has been reported that hyperglycemia participates in the onset and progressive impairment of diabetes mellitus, i.e., glucose toxicity theory. Namely, chronic hyperglycemia leads to decrease insulin secretion and further to decrease insulin sensitivity, and as a result, the blood glucose concentration is increased so that diabetes mellitus is self-exacerbated. Therefore, by treating
25 hyperglycemia, the aforementioned self-exacerbating cycle is interrupted so that the prophylaxis or treatment of diabetes mellitus is made possible.

Unfortunately, existing treatments do not succeed in restoring normoglycaemia in the long term, since beta-cell function declines over time. Moreover, there is presently no single drug able to reverse all aspects of the disease.

30 The progressive nature of type 2 diabetes means that many patients will eventually require a combination of oral hypoglycaemic medication, possibly together with insulin and/or exenatide injections. Anti-diabetic agents have been developed in order to counteract the main mechanisms involved in type 2 diabetes: insulin resistance (biguanides and thiazolidinediones) and insulin secretion (sulfonylureas, glinides, dipeptidylpeptidase-4 inhibitors, glucagon-like peptide 1

receptor agonists), agents that delay absorption of glucose by gastrointestinal tract or promote weight loss and newer agents that promote renal glucose excretion. However, most of these medications have been shown to have deleterious side effects such as weight gain, peripheral edema or congestive heart failure and there is a major problem with a loss of effectiveness of these agents with long-term use. Thus, despite the increasing number of therapeutic options for glycaemic control, there is a need for alternative and improved medications for the treatment of diabetes and related conditions.

SUMMARY OF THE INVENTION

The inventors identified a new target for treating diabetes, in particular Type 2 diabetes. They made the novel finding that ALMS1 (Alstrom syndrome protein 1) is involved in the regulation by insulin of glucose absorption by mature adipocytes through its binding interactions with key molecules involved in regulation of glucose. Briefly, when insulin binds its receptor, they showed that a protein complex forms around Alms1 (the ALMSome) and is activated, leading to H⁺ pump activation, GLUT4 receptor translocation and glucose absorption by adipocytes. They also showed that in the absence of Alms1, and thereby prevention of assembly of the ALMSome, glucose cannot be transported into the cells due to a failure of GLUT4 fusion with the cell membrane. Hence, they showed that modulation of ALMS1 complex formation can be used to regulate glucose transport and can thereby be used to circumvent insulin resistance, and treat Type 2 diabetes.

More particularly, the inventors identified two proteins involved in glucose transport regulation by ALMS1, namely TBC1D4 (TBC1 domain family member 4) and α PKC (PKC α or Protein Kinase C alpha type). More particularly, the binding sites of these two glucose regulating proteins on ALMS1 are so close that the simultaneous binding of both proteins is not possible due to steric hindrance. TBC1D4, through its interaction with proteins (i.e., Rab10, Rab14, etc) and ALMS1, regulates the translocation of GLUT4 receptors to the cell membrane. On the other hand, α PKC, when bound to ALMS1, blocks the TBC1D4 binding site and, thereby down-regulates the translocation of GLUT4 receptors to the cell membrane, reducing cellular glucose absorption. They further demonstrated that targeting the interaction of ALMS1 and α PKC is sufficient to trigger glucose absorption in the adipocytes irrespective of the presence of INS. Accordingly, a new therapeutic strategy revealed in this invention is to enhance cellular glucose absorption and reduce hyperglycaemia by blocking the binding of α PKC on ALMS1. Most preferably, the binding of α PKC on ALMS1 is inhibited in such a way that the binding of TBC1D4 on ALMS1 is unaffected or even enhanced.

Accordingly, the present invention relates to a molecule capable of preventing the binding of α PKC to ALMS1 for use for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia. It also relates to the use of such a molecule for the manufacture of a medicament for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia. It also relates to a method for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia, in a subject in need thereof, wherein a therapeutically effective amount of a molecule capable of preventing the binding of α PKC to ALMS1 is administered, thereby increasing the glucose absorption induced by insulin. In a preferred embodiment, the molecule does not interfere with the binding of TBC1D4 to ALMS1. Preferably, the molecule is selected from the group consisting of peptides or polypeptides or peptide mimetics, antibodies, fragments or derivatives thereof, aptamers, Spiegelmers, and chemical compounds. More preferably, the molecule is a peptide less than 50 amino acids, preferably less than 20 amino acids.

In a first preferred embodiment, the molecule is a peptide comprising an amino acid sequence of a fragment of ALMS1 (SEQ ID No 1). Preferably, the molecule is a peptide comprising an amino acid sequence of a fragment of ALMS1 including one or several of the residues which are predicted to mediate the interaction with α PKC, in particular one or several of the residues selected in the list consisting of E17, D58, S59, G62, H65, L66, Q736, T737, E738, D828, S829, T1088, D1089, A1169, Q1170, F2882, L2883, and E2884. In a very particular embodiment, the molecule is a peptide comprising or consisting of one of the following sequences:

- LDSDSHYGPQHLESIDD (SEQ ID No 5);
- DSHQTEETL (SEQ ID No 6);
- QQTLPESHLP (SEQ ID No 7);
- QALLDSHLPE (SEQ ID No 8);
- PADQMTDTP (SEQ ID No 9);
- HIPEEAQKVSAV (SEQ ID No 10);
- SCIFLEQ (SEQ ID No 11), and
- a fragment thereof comprising 6 contiguous amino acids.

In a second preferred embodiment, the molecule is a peptide comprising an amino acid sequence of a fragment of α PKC (SEQ ID No 4). Preferably, the molecule is a peptide comprising an

amino acid sequence of a fragment of α PKC including one or several of the residues which are predicted to mediate the interaction with ALMS1, in particular one or several of the residues selected in the list consisting of F114, D116, C118, L121, N138, Q142, I145, P148, G433, E545, S562, S566, F597, D601, W602, K604, E606, G620, T631, V664, and I667.

- 5 The present invention also relates to a method for identifying molecules suitable for use for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia, wherein the capacity of the molecule to prevent the binding of α PKC to ALMS1 is assayed and the molecules capable of preventing this binding are selected.
- 10 The method may additionally comprises a step in which the capacity of the selected molecule to interfere with the binding of TBC1D4 to ALMS1 is tested and wherein the molecules which do not interfere are selected. Preferably, the binding is determined in a cellular system responsive to insulin. Optionally, the binding is determined in presence and/or absence of insulin.

A further therapeutic strategy revealed in this invention is to enhance cellular glucose absorption by enhancing the binding of TBC1D4 on ALMS1. A further therapeutic strategy revealed in this invention is to enhance cellular glucose absorption by upregulating expression of ALMS1.

- Accordingly, the present invention further relates to a molecule capable of enhancing the binding of TBC1D4 on ALMS1 or increasing the expression of ALMS1 for use in treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia, in particular Type 2 diabetes. It also relates to the use of such a molecule for the manufacture of a medicament for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia, in particular Type 2 diabetes.
- 20 It also relates to a method for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia, in particular Type 2 diabetes, in a subject in need thereof, wherein a therapeutically effective amount of a molecule capable of enhancing the binding of TBC1D4 on ALMS1 or increasing the expression of ALMS1 is administered, thereby increasing the glucose absorption induced by insulin. In a preferred embodiment, the molecule also inhibits the binding of α PKC on ALMS1.
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- 30

The present invention also relates to a method for identifying molecules suitable for use for treating diabetes, wherein the capacity of the molecule to increase the expression of ALMS1 is assayed and the molecules capable of upregulating ALMS1 are selected. It further relates to

method for identifying molecules suitable for use for treating diabetes, wherein the capacity of the molecule to increase the binding of TBC1D4 to ALMS1 is assayed and the molecules capable of increasing this binding are selected. Optionally, the method further comprises determining the capacity of the molecule to prevent the binding of α PKC to ALMS1 is assayed and selecting the molecules capable of preventing this binding

DETAILED DESCRIPTION OF THE INVENTION

The inventors identified ALMS1 as the missing key player involved in regulation the insulin-mediated glucose uptake through GLUT4 sorting vesicles into adipocytes.

It has been now acknowledged that, even if adipose tissue is responsible of about 20 % of the glucose absorption, a dysfunction in this tissue can lead to diabetes occurrence. Therefore, any means capable of regulating the insulin-mediated glucose uptake into adipocytes should be able to delay, reverse, or prevent the occurrence of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia.

ALMS1 activity is downregulated by the binding of α PKC whereas it is activated by the binding of TBC1D4. It has also be shown that the binding sites of these two proteins on ALMS1 are so close that the simultaneous binding of both proteins is not allowed due to steric hindrance. Therefore, this regulation mechanism is a new target for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia and the inventors propose to use a molecule capable of preventing the binding of α PKC to ALMS1 for these therapeutic indications.

Definitions

ALMS1, Alstrom syndrome protein 1, is a protein encoded by the *ALMS1* gene. Mutations in the ALMS1 gene have been found to be causative for Alström syndrome. It is described in several databases, namely UniProt ID No Q8TCU4; Gene ID No 7840, HGNG ID No 428. Reference sequences are disclosed in Genbank under NM_015120.4 for mRNA and NP_055935.4 for protein. The protein sequence of human ALMS1 is disclosed in SEQ ID No 1.

TBC1D4 (TBC1 domain family member 4), also currently called As160, is supposed to act as a GTPase-activating protein for RAB2A, RAB8A, RAB10 and RAB14. It is described in several databases, namely UniProt ID No O60343, Gene ID No 9882, HGNG ID No 19165. Reference sequences are disclosed in Genbank under NM_014832.3 for mRNA and NP_055647.2 for

protein (for isoform 1, chosen as canonical sequences). The isoform 2, which differs from isoform by the missing of the amino acids in positions 678-740 and referenced in UniProt under No O60343-2, promotes insulin-induced glucose transporter SLC2A4/GLUT4 translocation at the plasma membrane, thus increasing glucose uptake. The protein sequence of human TBC1D4 (isoform 1) is disclosed in SEQ ID No 2. The protein sequence of human TBC1D4 (isoform 2) is disclosed in SEQ ID No 3.

Protein kinase C alpha type, also called α PKC, PKC-A or PKC-alpha, belongs to a family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. It is described in several databases, namely UniProt ID No P17252, Gene ID No 9393, HGNG ID No 5578. Reference sequences are disclosed in Genbank under NM_02737.2 for mRNA and NP_002728.1 for protein. The protein sequence of human α PKC is disclosed in SEQ ID No 4.

Screening Methods

The present invention relates to an *in vitro* or *ex vivo* method for identifying, screening or selecting a molecule capable of preventing the binding of α PKC to ALMS1. The method comprises determining the effect of molecule(s) on the binding of α PKC to ALMS1, and selecting the molecule(s) if the binding of α PKC to ALMS1 is decreased or prevented. Preferably, the method further comprises determining the effect of molecule(s) on the binding of TBC1D4 to ALMS1, and eliminating the molecule(s) if the binding of TBC1D4 to ALMS1 is decreased or prevented. Optionally, the method may comprise a step of selecting the molecule(s) if the binding of TBC1D4 to ALMS1 is increased or enhanced.

The present invention also relates to an *in vitro* or *ex vivo* method for identifying, screening or selecting a molecule capable of enhancing or increasing the binding of TBC1D4 to ALMS1. The method comprises determining the effect of molecule(s) on the binding of TBC1D4 to ALMS1, and selecting the molecule(s) if the binding of TBC1D4 to ALMS1 is increased or enhanced. Optionally, the method further comprises determining the effect of molecule(s) on the binding of α PKC to ALMS1, and selecting the molecule(s) if the binding of α PKC to ALMS1 is decreased or prevented.

In order to determine the effect of a molecule on the binding of α PKC and/or TBC1D4 to ALMS1, any technology known by the person skilled in the art can be carried out, in particular any method suitable for determining protein interactions. For example, recombinant or purified

native ALMS1 or α PKC can be bound to a surface plasmon resonance chip and the other molecule flowed over the chip to assess the binding affinity, for example in a Biacore (General Electric, USA) machine. The same approach can be used to measure the binding affinity of ALMS1 and TBC1D4 or of ALMS1 and α PKC.

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The effect of molecule(s) on the binding of α PKC and/or TBC1D4 to ALMS1 is determined by measuring the binding of α PKC and/or TBC1D4 to ALMS1 in absence and in presence of the tested molecule and by comparing the bindings of α PKC and/or TBC1D4 to ALMS1.

10 In addition, the screening method may comprise a preliminary step for selecting the molecule(s) capable of binding to ALMS1. Indeed, it could be advantageous that the molecule preventing the interaction between ALMS1 and α PKC acts directly on the ALMS1 binding site for α PKC.

Alternatively, the screening method may comprise a preliminary step for selecting the molecule(s) capable to bind to α PKC. Indeed, it could also be advantageous that the molecule
15 preventing the interaction between ALMS1 and α PKC acts directly on the α PKC binding site for ALMS1.

In addition, the screening method may comprise a preliminary step for selecting the molecule(s) capable to bind to TBC1D4.

20 In a preferred embodiment for identifying, screening or selecting a molecule capable of preventing the binding of α PKC to ALMS1, the screening method of the present invention further comprises determining the effect of the molecule(s), in particular the selected molecule(s), on the binding of TBC1D4 to ALMS1 and selecting the molecule(s) if the binding of TBC1D4 to ALMS1 is not decreased or prevented by the molecule(s). Even more, the method
25 may comprise a step of selecting the molecule(s) if TBC1D4 to ALMS1 is increased or enhanced by the molecule(s).

In a preferred embodiment for identifying, screening or selecting a molecule capable of enhancing the binding of TBC1D4 to ALMS1, the screening method of the present invention
30 further comprises determining the effect of the molecule(s), in particular the selected molecule(s), on the binding of α PKC to ALMS1 and selecting the molecule(s) if the binding of α PKC to ALMS1 is decreased or prevented by the molecule(s).

Due to the large size of the binding partners, in particular ALMS1 and TBC1D4, the inventors prefer using cellular systems for the screening methods. Preferably, the cellular system is a cellular system responsive to insulin. For instance, the cellular system could be selected among a mesenchymal cell line, a mesenchymal stem cell, an adipose mesenchymal stem cell, a pre-adipocyte and an adipocyte. Preferably, the cell is a human cell.

Then, the binding determinations can be carried in absence or presence of insulin, preferably in presence of insulin for the binding of α PKC to ALMS1 and in the presence insulin for the binding of TBC1D4 to ALMS1.

In a first aspect, immunoprecipitation assay using ALMS1 as bait can be carried, in particular as detailed in the experimental section. For instance, the assay can be carried out with cells, in particular adipocytes, cultured in absence and/or presence of insulin, preferably in absence of insulin. The molecules to be tested are added in the culture medium. Then, α PKC is immunodetected. Optionally, TBC1D4 can also be immunodetected. This method is disclosed in details in the Examples section.

In a preferred embodiment, the amount of α PKC bound to ALMS1 is determined and compared to the amount in absence of tested molecules, in particular in absence of insulin. If the amount of α PKC bound to ALMS1 decreases in presence of the tested molecule, then the molecule is selected.

The amount of TBC1D4 bound to ALMS1 is determined and compared to the amount in absence of tested molecules, in particular in presence of insulin or both in presence and absence of insulin. If the amount of TBC1D4 bound to ALMS1 decreases in presence of the tested molecule, then the molecule is rejected. If the amount of TBC1D4 bound to ALMS1 increases in presence of the tested molecule, then the molecule is selected.

In a second aspect, affinity purification coupled to mass spectrometry can be carried out, in particular after chemical crosslinking. For instance, cells may be cultured in a medium devoid of methionine and leucine but comprising photo-activable methionine and leucine. Then, cells are UV irradiated in order to stabilize protein complexes and protein complexes are analyzed by mass spectrometry.

Other methods are available to the person skilled in the art, e.g., Bimolecular fluorescence complementation, Tandem affinity purification, and the like. In particular, WO2012/117245

discloses a method for identifying molecules capable of preventing the interaction between two proteins: WO2012/117245 (i.e., for identifying small molecules). WO12174489 also discloses methods for developing molecules suitable for preventing interaction between two proteins.

5 In addition, suitable molecules can also be designed by molecular modelling. Such methods are for instance detailed in the Example section.

In a particular aspect, the present invention relates to a structural homology model of ALMS1 and its use in an *in silico* method to identify molecules able to inhibit or stimulate ALMSome function, in particular to inhibit the interaction between ALMS1 and α PKC and/or to increase the
10 interaction between ALMS1 and TBC1D4.

It also relates to a structural homology model of TBC1D4 and its use in an *in silico* method to identify molecules able to inhibit or stimulate ALMSome function, in particular to increase the interaction between ALMS1 and TBC1D4.

15 The present invention also relates to a method for identifying, screening or selecting a molecule capable of upregulating ALMS1 at the gene and protein level. The method comprises determining the effect of molecule(s) on the expression of ALMS1, and selecting the molecule(s) if the expression of ALMS1 is increased. In order to determine the effect of a molecule on the expression of ALMS1, any technology known by the person skilled in the art can be carried out.

20 Various techniques known in the art may be used to detect or quantify ALMS1 expression, including sequencing, hybridisation, amplification and/or binding to specific ligands (such as antibodies). Suitable methods include Southern blot (for DNAs), Northern blot (for RNAs), fluorescent in situ hybridization (FISH), gel migration, ELISA, radio-immunoassays (RIA) and immuno-enzymatic assays (IEMA).

25 By “increased”, “increase” or “enhance” is intended to refer to a binding increased by at least 10, 20, 30, 40, 50, 60, 70, 80 or 90 % when compared to the binding measured in absence of the tested molecule in the same conditions. By “decreased” or “decrease” is intended to refer to a binding decreased by at least 10, 20, 30, 40, 50, 60, 70, 80 or 90 % when compared to the
30 binding measured in absence of the tested molecule in the same conditions.

In addition, the screening methods of the present invention may comprise assay with animal models. Molecules to be tested may be administered to the animal models and the effect of the molecules on the glucose absorption or diabetes could be assessed. For instance, the animal

models could be mice or rat with insulin resistance, diabetes, or hyperglycemia. The effect of the molecule can be assessed by measuring the level of blood glucose.

Molecules

5 The molecules capable of preventing or blocking the binding of α PKC to ALMS1 can be any ligand capable of binding either α PKC or ALMS1 and, thereby preventing or blocking the binding of α PKC to ALMS1.

In a first aspect, the present invention relates to a molecule that prevents or blocks the binding of α PKC to ALMS1 by interacting with one or more of the ALMS1 residues selected in the list
10 consisting of E17, D58, S59, G62, H65, L66, Q736, T737, E738, D828, S829, T1088, D1089, A1169, Q1170, F2882, L2883, and E2884. In an alternative aspect, the present invention relates to a molecule that prevents or blocks the binding of α PKC to ALMS1 by interacting with one or more of the α PKC residues selected in the list consisting of F114, D116, C118, L121, N138, Q142, I145, P148, G433, E545, S562, S566, F597, D601, W602, K604, E606, G620, T631,
15 V664, and I667.

The molecules capable of enhancing or increasing the binding of TBC1D4 to ALMS1 can be any ligand capable of binding either TBC1D4 or ALMS1 and, thereby enhancing or increasing the binding of TBC1D4 to ALMS1.

20 In a first aspect, the present invention relates to a molecule that enhances or increases the binding of TBC1D4 to ALMS1 by interacting with one or more of the ALMS1 residues selected in the list consisting of H65, L66 and S2879. In an alternative aspect, the present invention relates to a molecule that enhances or increases the binding of TBC1D4 to ALMS1 by interacting with one or more of the TBC1D4 residues selected in the list consisting of G75, A76, P77, A78, R80, E81,
25 V82, and I83.

The present invention relates to such molecules, a pharmaceutical composition comprising such molecules, and the use of such molecules as a drug or for the manufacture of a drug.

30 The molecules can be peptides or polypeptides or peptide mimetics, antibodies, fragments or derivatives thereof, aptamers, Spiegelmers, or chemical compounds. The molecules can be selected by the screening methods known in the art or as detailed above and can be designed by any convenient *in silico* modeling method.

In a preferred embodiment, the molecule is a peptide or polypeptide. Preferably, the peptide may have between 5 and 50 amino acids. More preferably, it has between 5 and 20 amino acids. More preferably, the peptide or polypeptide comprises less than 50 amino acids, more preferably less than 40, 30, 20, 15 or 10 amino acids.

5 In a first aspect, the molecule is a peptide or polypeptide comprising an amino acid sequence of a fragment of ALMS1 (SEQ ID No 1). In a preferred embodiment, the molecule is a peptide or polypeptide comprising an amino acid sequence of a fragment of ALMS1 including one or several of the residues which are predicted to mediate the interaction with α PKC. In particular, these residues are selected in the list consisting of E17, D58, S59, G62, H65, L66, Q736, T737,
 10 E738, D828, S829, T1088, D1089, A1169, Q1170, F2882, L2883, and E2884. More preferably, these residues are selected in the list consisting of D58, S59, G62, H65, L66, Q736, T737, E738, D828, S829, T1088, D1089, A1169, Q1170, F2882, L2883, and E2884. D58, S59, G62, H65 and L66 define a first interaction segment. T737 and E738 define a second interaction segment. D828 and S829 define a third interaction segment. T1088 and D1089 define a fourth interaction
 15 segment. A1169 and Q1170 define a fifth interaction segment. F2882, L2883 and E2884 define a sixth interaction segment.

In a very particular aspect, the peptide or polypeptide comprises or consists of one of the following sequences:

- LDSDSHYGPQHLESIDD (SEQ ID No 5), targeting the first interaction segment;
- 20 - DSHQTEETL (SEQ ID No 6), targeting the second interaction segment;
- QQTLPESHLP (SEQ ID No 7);
- QALLDSHLPE (SEQ ID No 8), targeting the third interaction segment;
- PADQMTDTP (SEQ ID No 9), targeting the fourth interaction segment;
- HIPEEAQKVSAV (SEQ ID No 10), targeting the fifth interaction segment;
- 25 - SCIFLEQ (SEQ ID No 11), targeting the sixth interaction segment, and
- a fragment thereof comprising 6 contiguous amino acids.

In a second aspect, the molecule is a peptide or polypeptide comprising an amino acid sequence of a fragment of α PKC (SEQ ID No 4). In a preferred embodiment, the molecule is a peptide or polypeptide comprising an amino acid sequence of a fragment of α PKC including one or several
 30 of the residues which are predicted to mediate the interaction with ALMS1. In particular, these residues are selected in the list consisting of F114, D116, C118, L121, N138, Q142, I145, P148, G433, E545, S562, S566, F597, D601, W602, K604, E606, G620, T631, V664, and I667. F114, D116, C118 and L121 may define a first interaction segment. N138, Q142, I145 and P148 may define a second interaction segment. E545, S562 and S566 may define a third interaction

segment. F597, D601, W602, K604, and E606 define a fourth interaction segment. V664 and I667 may define a fifth interaction segment.

Optionally, the peptide or polypeptide may comprise one, two, three, four or five amino acid substitution in comparison to the reference sequence, i.e., SEQ ID No 1 for peptides derived from ALMS1, SEQ ID No 4 for peptides derived from α PKC, and SEQ ID No 2 or 3 for peptides derived from TBC1D4.

The peptide or polypeptide may further comprise a moiety facilitating its cellular uptake or entry, in particular a PTD (protein transduction domain). PTD generally comprises a certain amino acid sequence of 10 to 20 amino acids (Matsushita and Matsui, (2005), J Mol Med 83, 324-328; Vivès et al, Biochimic et Biophysica Acta, 2008, 1786, 126-138). PTD is mainly composed of basic amino acids such as arginine or lysine, and representative examples of the PTD include arginine rich peptides such as poly R₈ (RRRRRRRR) or (RRPRRPRRPRRPRR), antennapedia or penetratin peptide such as (RQIKIWFQNRRMKWKK) or HIV-Tat (YGRKKRRQRRR).

In a particular aspect, the molecule is an antibody, fragment or derivative thereof.

The peptide or polypeptide can be made of natural amino acids and/or unnatural amino acids. By “unnatural amino acids” is intended an analog or derivative of a natural amino acid (i.e., Ala, Val, Gly, Leu, Ile, Lys, Arg, Glu, Gln, Asp, Asn, His, Tyr, Phe, Trp, Ser, Pro, Thr, Cys, Met). They present a modified side chain, e.g. shorter, longer or with different functional groups.

Isomers D and L are contemplated, in particular because isomers D are not sensible to proteases. In addition, modifications in some or all peptide bounds are also contemplated in order to increase the proteolysis resistance, in particular by (-CO-NH-) by (-CH₂-NH-), (-NH-CO-), (-CH₂-O-), (-CH₂-S-), (-CH₂-CH₂-), (-CO-CH₂-), (-CHOH-CH₂-), (-N=N-), and/or (-CH=CH-).

The peptide can present a carboxylic C terminal end (-COO⁻) and an amide one (-CONH₂).

The peptide can also be D-retro-inverso sequence of a peptide as disclosed herein. The N terminal can be modified, especially with an acetyl radical. Optionally, the peptide or polypeptide can be PEGylated in order to increase the stability. Alternatively, the peptide can be modified to become a stapled peptide. The term “stapled peptide” as used herein refers to artificially modified peptide in which the structure is stabilized with one or more artificial molecular bridging (cross links) that connects adjacent turns of α -helices in the peptide. The modalities for preparing stapled peptides have been reviewed extensively for instance in Verdine & Hilinski (2012, Methods Enzymol, 503, 3-33), WO10033617 and WO10011313, the disclosure of which being incorporated herein by reference).

The present invention further relates to a pharmaceutical composition comprising a peptide as defined above and a pharmaceutically acceptable carrier/excipient. It also relates to a peptide as defined above for use as a drug or to the use of a peptide as defined above for the manufacture of a medicament.

5

In an alternative embodiment, the molecule is an antibody, a fragment thereof or a derivative thereof. As used herein, the terms “antibody” and “immunoglobulin” have the same meaning and are used indifferently in the present invention. The term “antibody” refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen-binding site that immunospecifically binds an antigen. Antibodies include any kind of antibodies, preferably monoclonal. They can be for instance IgG (immunoglobulin G) or VHH (heavy chain variable domain antibody from camelids). Antibodies fragments or derivatives thereof include Fab, Fab', F(ab')₂, scFv, (scFv)₂, dAb, complementarity determining region (CDR) fragments, linear antibodies, single-chain antibody molecules, minibodies, diabodies, and multispecific antibodies formed from antibody fragments.

15

Antibodies, fragments or derivatives can block the interaction between ALMS1 and α PKC. Preferably, they have no effect on the interaction between ALMS1 and TBC1D4 or have an enhancing effect on the interaction.

20

In a first embodiment, the antibody is specific for ALMS1. In particular, the epitope of the antibody comprises one or several of the ALMS1 residues involved in the interaction with α PKC, in particular one or several residues selected in the list consisting of E17, D58, S59, G62, H65, L66, Q736, T737, E738, D828, S829, T1088, D1089, A1169, Q1170, F2882, L2883, and E2884.

25

Alternatively, the antibody is specific for α PKC. In particular, the epitope of the antibody comprises one or several of the α PKC residues involved in the interaction with ALMS1, in particular one or several residues selected in the list consisting of F114, D116, C118, L121, N138, Q142, I145, P148, G433, E545, S562, S566, F597, D601, W602, K604, E606, G620, T631, V664, and I667.

30

Such antibodies can be produced by immunizing non-human mammals with immunogenic peptides or proteins comprising one or several residues identified as involved in the interaction between ALMS1 and α PKC. Alternatively, library of antibodies can be provided and screened. Produced antibodies, fragments or derivatives are then screened for their capacity to bind one of the interacting partners and/or their capacity to prevent, inhibit or block the interaction between ALMS1 and α PKC. In addition, as previously specified, antibodies, fragments or derivatives can

be further screened for their capacity to modulate the interaction between TBC1D4 and ALMS1, and selected if they increase or enhance the interaction.

Antibodies, fragments or derivatives can enhance the interaction between ALMS1 and TBC1D4.

5 Preferably, they have a blocking effect on the interaction between ALMS1 and α PKC.

In a first embodiment, the antibody is specific for ALMS1. In particular, the epitope of the antibody comprises one or several of the ALMS1 residues involved in the interaction with TBC1D4, in particular one or several residues selected in the list consisting of H65, L66 and S2879.

10 Alternatively, the antibody is specific for TBC1D4. In particular, the epitope of the antibody comprises one or several of the TBC1D4 residues involved in the interaction with ALMS1, in particular one or several residues selected in the list consisting of G75, A76, P77, A78, R80, E81, V82, and I83.

Such antibodies can be produced by immunizing non-human mammals with immunogenic
15 peptides or proteins comprising one or several residues identified as involved in the interaction between ALMS1 and TBC1D4. Alternatively, library of antibodies can be provided and screened. Produced antibodies, fragments or derivatives are then screened for their capacity to bind one of the interacting partners and/or their capacity to enhance or increase the interaction between ALMS1 and TBC1D4. In addition, as previously specified, antibodies, fragments or
20 derivatives can be further screened for their capacity to modulate the interaction between α PKC and ALMS1, and selected if they decrease or block the interaction.

The preparation of monoclonal or polyclonal antibodies is well known in the art, and any of a large number of available techniques can be used (see, e.g., Kohler & Milstein, Nature 256:495-
25 497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); Cole et al., pp. 77-96 in Monoclonal Antibodies and Cancer Therapy (1985)). Techniques for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce antibodies to desired polypeptides. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized, chimeric, or similarly-modified antibodies. Alternatively, phage display
30 technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., Nature 348:552-554 (1990); Marks et al., Biotechnology 10:779-783 (1992)).

For aptamers and Spiegelmers, similar methods can be used in order to select aptamers and Spiegelmers. These methods are well-known by the person skilled in the art.

As used here, the term “aptamer” means a molecule of nucleic acid or a peptide able to bind ALMS1, α PKC or TBC1D4. It refers to a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide or oligopeptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity.

Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX) of a random sequence library, as described in Tuerk C. and Gold L., Science, 1990, 249(4968):505-10. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., Clin. Chem., 1999, 45(9):1628-50. Peptide aptamers consist of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al., Nature, 1996,380, 548-50).

Spiegelmers have been disclosed for instance in WO 98/08856. They are molecules similar to aptamers. However, spiegelmers consist either completely or mostly of L-nucleotides rather than D-nucleotides in contrast to aptamers. Otherwise, particularly with regard to possible lengths of spiegelmers, the same applies to spiegelmers as outlined in connection with aptamers.

Chemical compounds refers to a molecule of less than about 1500 Daltons, 1000 Daltons, 800 Daltons, or even less than about 500 Daltons, in particular organic or inorganic compounds. Structural design in chemistry should help to find such a molecule. The molecule may have been identified by a screening method disclosed in the present invention.

Synthetic compound libraries are commercially available from a number of companies including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, N. J.), Brandon Associates (Merrimack, N.H.), and Microsource (New Milford, Conn.). Combinatorial libraries are available or can be prepared according to known synthetic techniques. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from e.g., Pan Laboratories (Bothell, Wash.) and MycoSearch (NC), or are readily producible by methods well known in the art.

Additionally, natural and synthetically produced libraries and compounds can be further modified through conventional chemical and biochemical techniques.

The molecule can be linked, covalently or not, to a moiety targeting the relevant tissues, preferably the adipose or to a moiety facilitating the entrance of the molecule into cells.

5 *Therapeutic indications*

The inventors propose to use the molecules as disclosed herein for increasing the glucose uptake, in particular by adipocytes, thereby regulating or controlling the blood glucose level. Then, the molecules are suitable for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin
10 resistance, hyperglycemia, obesity, and hyperinsulinaemia.

Diabetes mellitus is characterized by hyperglycemia. More particularly, type 2 diabetes is characterized by hyperglycemia and insulin resistance. Obesity is thought to be the primary cause of type 2 diabetes in people who are genetically predisposed to the disease. Diabetic retinopathy, diabetic neuropathy, diabetic nephropathy are well-known disorders associated with
15 diabetes and insulin resistance.

Then, decreasing the glycemia by increasing the glucose uptake could treat or delay the progression or onset of these diseases.

The present invention also relates to the molecules according to the invention for use for
20 reducing the dose of insulin or stopping the insulin treatment when used for treating diabetes in a subject, to the use of the molecules according to the invention for the manufacture of a medicament for reducing the dose of insulin or stopping the insulin treatment when used for treating diabetes in a subject, or to a method for treating diabetes in a subject, wherein a therapeutically effective amount of a molecule according to the invention is administered to a
25 subject with a decreased dose of insulin or in absence of insulin treatment. More generally, it can be used to lower the doses of anti-diabetic drugs.

By “treat” or “treatment” is intended that the disease is cured, alleviated or delayed. It includes the preventive or curative treatment. The term treatment designates in particular the correction, retardation, or reduction of an impaired glucose homeostasis. The term “treatment” also
30 designates an improvement in glucose uptake (e.g., capture of glucose by adipocytes). Within the context of the invention, the terms “controlling the blood glucose level” or “the control of blood glucose level” refer to the normalization or the regulation of the blood or plasma glucose level in a mammalian subject having abnormal levels (i.e., levels that are below or above a known

reference, median, or average value for a corresponding mammalian subject with a normal glucose homeostasis).

The present invention relates to the pharmaceutical or veterinary use of the molecule. Accordingly, the subject may be any mammal, preferably a human subject, such as an adult or a children. In a particular embodiment, the subject is a subject suffering of obesity. Optionally, the subject has no detectable anti-islet antibodies, and ultrasonography revealed no pancreatic abnormalities. In the context of a veterinary application, the subject can be an animal, preferably a mammal, in particular a pet animal such as a dog, a cat or a horse.

The molecules according to the invention can be used in combination with one or more additional active drugs, preferably anti-diabetic drugs, in particular for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia.

Therefore, the present invention also relates to a pharmaceutical composition comprising a molecule according to the present invention and one or more additional active drugs, preferably an anti-diabetic drug.

It further relates to a product or kit containing a molecule according to the invention and one or more additional active drugs, preferably anti-diabetic drugs, as a combined preparation for simultaneous, separate or sequential use, or a combined preparation which comprises a molecule according to the invention and one or more additional active drugs, preferably anti-diabetic drugs, for simultaneous, separate or sequential use, in particular for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia.

It relates to a molecule according to the invention for use for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia in combination with one or more additional active drugs, preferably anti-diabetic drugs.

It further relates to the use of a molecule according to the invention and one or more additional active drugs, preferably anti-diabetic drugs, for the manufacture of a medicament, in particular treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic

retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia.

Finally, it relates a method for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin
5 resistance, hyperglycemia, obesity, and hyperinsulinaemia, wherein a therapeutic effective amount of a molecule according to the invention is administered in combination with a therapeutic or sub-therapeutic effective amount of one or more additional active drugs, preferably anti-diabetic drugs. By “sub-therapeutic” is intended to refer to an amount can be for instance 90, 80, 70, 60, 50, 40, 30, 20 or 10 % of the conventional therapeutic dosage (in
10 particular for the same indication and the same administration route).

In particular, the additional active drug is a drug used for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia. For instance, the
15 additional drug can be an anti-diabetic drug such as a hypoglycemic agent or an antihyperglycemic agent. It may be selected in the non-exhaustive list comprising insulin, metformin, sulfonylureas such as tolbutamide, acetohexamide, tolazamide, chlorpropamide, glyburide (also called glibenclamide), glimepiride, glipizide, glicazide, glycopyramide and gliquidone, alpha-glucosidase inhibitors such as acarbose, miglitol and voglibose,
20 thiazolidinediones such as pioglitazone and rosiglitazone, a meglitinide such as repaglinide and nateglinide, incretin mimetics, glucagon-like peptide analogs and agonists such as exenotide, taspoglutide and liraglutide, dipeptidyl peptidase-4 inhibitors such as vildagliptin, sitagliptin, saxagliptin, linagliptin,alogliptin, and septagliptin, amylin analogs such as pamlintide, glycourics such as canagliflozin and dapagliflozin, or any combination thereof.

25 The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc.

The pharmaceutical or therapeutic compositions of the invention can be formulated for a topical,
30 oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like.

The molecule used in the pharmaceutical composition of the invention is present in a therapeutically effective amount. The term “therapeutically effective amount” as used in the present application is intended an amount of therapeutic agent, administered to a patient that is

sufficient to constitute a treatment of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia as defined above.

The pharmaceutical composition comprising the molecule is formulated in accordance with standard pharmaceutical practice (Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York) known by a person skilled in the art.

For oral administration, the composition can be formulated into conventional oral dosage forms such as tablets, capsules, powders, granules and liquid preparations such as syrups, elixirs, and concentrated drops. Non toxic solid carriers or diluents may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. For compressed tablets, binders, which are agents which impart cohesive qualities to powdered materials, are also necessary. For example, starch, gelatine, sugars such as lactose or dextrose, and natural or synthetic gums can be used as binders. Disintegrants are also necessary in the tablets to facilitate break-up of the tablet. Disintegrants include starches, clays, celluloses, algin, gums and crosslinked polymers. Moreover, lubricants and glidants are also included in the tablets to prevent adhesion to the tablet material to surfaces in the manufacturing process and to improve the flow characteristics of the powder material during manufacture. Colloidal silicon dioxide is most commonly used as a glidant and compounds such as talc or stearic acids are most commonly used as lubricants.

For transdermal administration, the composition can be formulated into ointment, cream or gel form and appropriate penetrants or detergents could be used to facilitate permeation, such as dimethyl sulfoxide, dimethyl acetamide and dimethylformamide.

For transmucosal administration, nasal sprays, rectal or vaginal suppositories can be used. The active compound can be incorporated into any of the known suppository bases by methods known in the art. Examples of such bases include cocoa butter, polyethylene glycols (carbowaxes), polyethylene sorbitan monostearate, and mixtures of these with other compatible materials to modify the melting point or dissolution rate.

Pharmaceutical compositions according to the invention may be formulated to release the active drug substantially immediately upon administration or at any predetermined time or time period after administration.

Pharmaceutical compositions according to the invention can comprise one or more molecule of the present invention associated with pharmaceutically acceptable excipients and/or carriers.

These excipients and/or carriers are chosen according to the form of administration as described above.

In a particular embodiment, the pharmaceutical composition according to the invention comprises 0.001 mg to 10 g of the molecule of the invention. Preferably, pharmaceutical composition according to the invention comprises 0.01 mg to 1 g of the molecule of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Metabolic characterization of the *Alms^{foz/foz}* mice

- 10 (A) Mean body weight of WT and *Alms^{foz/foz}* male mice (n = 6-8 mice per genotype). (B) Photograph of visceral adipose tissue from WT and *Alms^{foz/foz}*. Scale bar: 25μM. (C) Insulin tolerance test (I.T.T.) performed on WT and *Alms^{foz/foz}* mice and the corresponding histogram showing the Area under the curve (A.U.C.) for each genotype (n = 6-8 mice per group). p<0.001). (D) Mean body weight of WT and *Alms^{foz/foz}* male mice (n = 6-8 mice per genotype).
15 (E) Photograph of visceral adipose tissue from corresponding WT and *Alms^{foz/foz}*. Scale bar: 25μM. (F) Insulin tolerance test performed on WT and *Alms^{foz/foz}* mice and the corresponding histogram showing the A.U.C. for each genotype (n = 6-8 mice per group). *** stands for p-value <0.001. (G) Immunoblots for the indicated proteins in insulin sensitive tissues from non-obese WT and *Alms^{foz/foz}* mice. (H) Results of radioactive counts in different target tissues after
20 injection of radioactive deoxyglucose to WT and *Alms^{foz/foz}* mice (n = 5 mice per genotype). * stands for p-value = 0.05.

Figure 2. ALMS1 silencing effect in human mature adipocytes

- (A) Photographs showing the lack of absorption of 2-NBDG (green) in control (shCTRL shRNA) or ALMS1-deprived adipocytes (ALMS1 shRNA) silenced mature adipocytes in
25 absence of INS. (B) Photographs depicting lack of absorption of 2-NBDG in ALMS1 shRNA compared to CTRLshRNA. Nuclei were counterstained with DAPI, DIC: Differential Interference Contrast pictures. (C) 3D images of CTRLshRNA or ALMS1shRNA mature adipocytes stained for intracellular Triglycerides (TG), plasma membrane in red (PM) and nuclei in blue (DAPI). (D) Measurements of fluorescent levels correlating with amounts of intracellular
30 TG in mature adipocytes (n = 16 wells per condition measured) * p-value = 0.05. (E-F) Immunodetection of AKT and pS473-AKT in CTRLshRNA and ALMS1shRNA treated mature adipocytes in presence and absence of INS. (G) 3D images of CTRLshRNA and ALMS1shRNA mature adipocytes showing cellular localization of Insulin receptor (IR in red) and GLUT4 (in green) in absence of Ins. Cut-view images displaying the dynamics of GLUT4 localization in

absence of Ins. (H), after 30 min. INS. stimulation (I) and with 30 min INS. stimulation followed by 2 hours of absence of INS. (J) in CTRLshRNA and ALMS1shRNA mature adipocytes. Scale bars: 25µm in A, B, C and 5µm in G-J.

Figure 3. Predicted interaction sites on ALMS1 protein and modelling of its partner

5 **TBC1D4.** (A) Predicted 3D structure of the ALMS1 protein with helices and loops. (B) Predicted 3D structure of the ALMS1 protein with the potential interacting sites represented by red dots. (C) Primary sequence of TBC1D4 protein with indicated localization of binding sites or interacting domains. (D) Predicted 3D structure of the TBC1D4 protein.

Figure 4. ALMS1 is required for TBC1D4 cellular trafficking

10 (A) *In silico* predicted 3D structure showing spatial interaction between ALMS1 and TBC1D4 with an enlarged view of the interaction site highlighting the predicted interacting amino acids residues (L66, Y61 and S2879) of the ALMS1 protein. (B) 3D image from immunostained mature adipocytes depicting co-localization of TBC1D4 (green) and ALMS1 (red). Nuclei were counterstained with DAPI (blue). (C-D) Immunoblots for the indicated proteins on cell lysates
15 (50µg total protein loaded per lane) for CTRLshRNA and ALMS1shRNA mature adipocytes treated with or without insulin. 3D images of immunofluorescence experiments performed on either CTRLshRNA or ALMS1shRNA or TBC1D4shRNA mature adipocytes depicting cellular localization of GLUT4 in absence of Insulin (-INS) (E) or in the presence of INS. (F). PM: Plasma membrane and nuclei counterstained with DAPI. 3D images of immunofluorescence
20 experiments performed on either CTRLshRNA or ALMS1shRNA adipocytes showing cellular localization of GLUT4 (green) and TBC1D4 in absence of INS (G) or when treated 30 min. with INS. (H). Scale bars: 10µm.

Figure 5. TBC1D4 is not the sole interacting partner of ALMS1 playing a role in the adipocyte biology

25 (A-C) Photographs showing absorption of 2-NBDG in either CTRLshRNA or ALMS1shRNA or TBC1D4shRNA deprived adipocytes after 30 min Ins. stimulation. (D-F) 3D images obtained using non-permeabilized fixated mature adipocytes stimulated with INS. following immunodetection of GLUT4 membrane bound (green). Plasma membrane (PM) was stained with Image-iT (red) and nuclei were counterstained with DAPI. (G) Immunoblots of 2 proton
30 pumps subunits (ATP6V0D1 and ATP6V1A) identified by mass spectrometry in the IP experiments using ALMS1 as bait (Fig. S4), αPKC, GLUT4 and β-Tubulin in cellular extracts from white adipose tissue (WAT) and kidney. 50µg total protein loaded per lane. (H) Photograph of Duolink positive signal detected in adipocytes using antibodies against ALMS1 and ATP6. (I) Immunofluorescence pictures showing cellular localizations of ATP6V0D1 and ALMS1 and

merged in mature adipocytes upon INS. stimulation. (J) *In silico* predicted binding sites of TBC1D4 (red) and PKC (yellow) which are only 20 Angstroms away from each other in the ALMS1 3D structure. (K-L) Immunodetections of α PKC, TBC1D4 and α -Actinin in immunoprecipitates using ALMS1 as bait in adipocytes cultured in absence or presence of INS.

5 **Figure 6. Restoring acidification in ALMS1-deprived adipocytes reinstate glucose absorption**

(A-B) Time lapse pictures were performed on either control or ALMS1-deprived Acridine orange stained adipocytes stimulated with INS. (C-D) Time lapse pictures were performed on either control or ALMS1-deprived Acridine orange stained adipocytes stimulated with an
10 electroneutral K^+/H^+ exchange ionophore, Nigericin (NIG.). (E) Top to bottom: Scanning electron microscopy (SEM) pictures of control adipocytes stimulated either without Ins. or with INS. or with NIG. White arrows show swelled vesicles. (F) Corresponding Transmitted Electron microscopy (TEM) pictures shown in (E) showing vesicles fusion with the plasma membrane in presence of INS. and NIG. (G) Top to bottom: SEM pictures of ALMS1-deprived adipocytes
15 stimulated either without INS or with INS. or with NIG. (H) Corresponding TEM pictures shown in (G) showing vesicles fusion with the plasma membrane only in presence of NIG. (I) Photographs showing the intracellular content of 2-NBDG (green) in control mature adipocytes either in absence of INS. (top panel) or after 30 minutes INS. stimulation (middle panel) or after 30 min. NIG. Stimulation (bottom panel). (J) Photographs showing the intracellular content of 2-
20 NBDG (green) in ALMS1-deprived mature adipocytes either in absence of INS. (top panel) or after 30 minutes INS. stimulation (middle panel) or after 30 min. NIG. stimulation (bottom panel). Scale bars: 20 μ m except for F and H: 500nm.

Figure 7. GLUT4 trafficking requires ALMSome protein complex

(A) 3D images obtained using non-permeabilized fixated mature adipocytes stimulated with NIG.
25 following immunodetection of GLUT4 membrane bound (green). Plasma membrane (PM) was stained with Image-iT (red) and nuclei were counterstained with DAPI. (B) Photographs showing intracellular TG content 24 hrs. after NIG. treatment. (C) Schematic representation of ALMS1 cellular localization and protein partner in absence of INS. stimulation in mature adipocyte. (D) Schematic representation of ALMS1 dynamics and protein partners after INS.
30 stimulation in mature adipocyte.

Figure 8. Glucose absorption is triggered in absence of INS through specific interference of α PKC binding site in the ALMSome

(A) Photographs showing absorption of 2-NBDG in presence or absence of INS in adipocytes infected with either CTRL lentiviral particles or α PKC domain carrying lentiviral particles. (B)

Quantification of intracellular glucose analogue 2-NB in presence or absence of INS in adipocytes infected with either CTRL lentiviral particles or α PKC domain carrying lentiviral particles. (n = 8 per group).

Figure 9. min- α PKC-FLAG construct characterization in adipocytes

- 5 Top panel: Immunodetection of min- α PKC-FLAG using an anti-FLAG antibody in mature adipocytes 48 hours post lentiviral infection. 2nd and 3rd panels: 3D image of the adipocyte showing the perinuclear localization of min- α PKC-FLAG. Last panel: Schematic representation of the experimental approaches used to assess the effect of min- α PKC-FLAG on glucose absorption.

10

EXAMPLES

- 15 Alström syndrome (ALMS) is a rare autosomal recessive disorder characterized by several clinical features including obesity and early-onset diabetes. It originates due to mutations in the ALMS1 gene coding for a protein of 460 kDa.

The function of the ALMS1 gene and how it causes the Alström syndrome phenotype has hitherto been unknown, with studies into its function being impeded by the extremely large size of the encoded protein and its low levels of expression.

- 20 Alström syndrome (ALMS) is a rare monogenic childhood obesity syndrome for which there is only one causative mutated gene identified to date, the *ALMS1* gene. ALMS is classified as a member of the ciliopathy disorders that includes Bardet Biedl syndrome, a group of syndromic disorders originating from mutations in the large number of different proteins that together play a critical role in primary cilium function. Alms1 encodes the 461 kDa ALMS1 protein that was
25 originally described to bear a purely centriolar localization, although more recent data has also suggested a cytoplasmic localization of ALMS1.

- ALMS is clinically identified by collective multisystem phenotype thought to reflect the ubiquitous tissue expression of ALMS1, closely mimicking many of the phenotypic features of BBS. Common clinical features of ALMS include retinal degeneration, hearing loss, childhood
30 obesity, early-onset type 2 diabetes mellitus (T2DM) dilated cardiomyopathy, renal and hepatic dysfunction, hypothyroidism, short stature, hyperlipidemia, and organ fibrosis. Children with ALMS develop obesity in early childhood that is associated with early onset of T2DM at around 16 years of age with a much higher overall prevalence of early onset T2DM in ALMS than seen with other childhood obesity syndromes resulting in a similar body mass index (BMI) including

BBS. The reason for this predilection for T2DM in children with ALMS that is out of proportion to their degree of obesity has remained elusive.

The inventors investigated the role of the ALMS1 protein during the adipogenic differentiation process and found that the ALMS1 protein expression levels increased during adipogenesis.

5 ALMS1 suppression, in adipogenic differentiating mesenchymal stem cells, inhibited the anti-adipogenic cascades but surprisingly was not favoring adipogenesis.

In addition, the inventors showed the ALMS1 protein complex is also required in mature adipocytes for efficient GLUT4 retention in its insulin-responsive compartment and its ability to fuse with the plasma membrane in response to insulin stimulation. Inactivation of ALMS1
10 decreased the amount of glucose able to be absorbed by mature adipocytes upon insulin stimulation, therefore contributing to hyperglycaemia and the onset of diabetes.

Previous studies in the spontaneous mutant *Alms1^{foz/foz}* and genetrapped *Alms1* knockout murine ALMS models confirmed that these mice, similarly to affected human children, develop obesity in early adolescence due to hyperphagia, and also exhibit impaired glucose tolerance,
15 hyperinsulinemia and islet hypertrophy, consistent with severe insulin resistance, although the tissue origin or mechanism for this insulin resistance has previously not been characterised. Previously published studies of *in vitro* studies on the murine 3T3-L1 fibroblast cell line showed that inhibition of ALMS1 gene expression resulted in mild impairment of adipogenesis but was reported to have no effect on the insulin signaling pathway in the resulting mature adipocytes as
20 measured by insulin-mediated AKT phosphorylation. This data led directly away from the invention presented here that *Alms1* does indeed play a critical hitherto unrecognized role in the insulin signaling pathway and in GLUT4 mediated glucose transport.

Indeed, despite the previously published contrary data, the inventors when carefully studying the phenotype of the Fat Aussie murine ALMS model (*Alms1^{foz/foz}*) identified that insulin resistance
25 in this model preceded rather than followed the development of obesity. They further identified the adipose tissue as the specific site driving the insulin resistance and subsequent development of glucose intolerance and T2DM in ALMS. They confirmed that insulin signaling in *Alms1^{foz/foz}* adipocytes was intact all the way down to phosphorylation of TBC1D4, the last known member of the insulin-mediated glucose uptake pathway but then through a subsequent series of
30 investigations identified a protein complex they termed the *Almsome*, composed of several key proteins that associate with ALMS1 and which together are required for the tethering and fusion of the GLUT4 vesicles to the adipocyte plasma membrane (PM) in response to insulin signaling. The *Almsome* thereby represents the hitherto unidentified ultimate step in insulin-mediated glucose uptake into adipocytes, with insulin resistance in ALMS due to disruption of *Almsome*

function leading to failure of GLUT4 membrane fusion and thereby a block to adipocyte glucose transport.

EXAMPLE 1

5 ***Alms1*^{foz/foz} mice display severe specific adipose tissue insulin resistance even in the absence of obesity**

Animal husbandry

Alms1^{foz/foz} mice and *Alms1*^{+/+} (WT) littermates were maintained on a C57BL/6J background in the animal facility on a 12 hourly light/dark cycle. Mice had free access *ad libitum* to water and
10 either normal chow containing 5.4% fat, energy content 12 MJ/kg (Gordon's rat and mouse maintenance pellets, Gordon's specialty stockfeeds, Australia) or high fat diet (HFD) containing 23% Fat, High Simple carbohydrate, 0.19% cholesterol, energy content 20 MJ/kg (SF03-020, Specialty feeds, Australia). Primers flanking the *foz* mutation were used for PCR genotyping: forward ACA ACT TTT CAT GGC TCC AGT (SEQ ID No 13); reverse TTG GCT CAG AGA
15 CAG TTG AAA (SEQ ID No 14).

Six month old obese and young (< 60days old) nonobese *Alms1*^{foz/foz} mice and wildtype (WT) littermates were used to investigate what primary metabolic impairment leads *Alms1*^{foz/foz} mice to develop T2DM. Six month old *Alms1*^{foz/foz} mice were obese with an average body weight of 45.5g ± 1.7g compared to 26.4g ± 1.3g for WT littermates (Fig. 1A) and as previously shown
20 had fasting hyperglycaemia and impaired glucose tolerance with elevated HOMA scores. An insulin tolerance test (ITT) showed that unlike WT (Fig 1B) and heterozygous littermates, glycaemia in obese *Alms1*^{foz/foz} mice was unresponsive to insulin administration (Fig 1B), even when doses of insulin as high as 20U/kg were administered. (Fig. 1C). Obesity of *Alms1*^{foz/foz} mice was due to severe adipocyte hypertrophy (Fig. 1B) rather than the adipocyte hyperplasia
25 more typically seen in obese BBS mice. To determine what the primary defect was that was causing the glucose intolerance in *Alms1*^{foz/foz} mice, young lean *Alms1*^{foz/foz} mice were studied to remove the confounding effect of obesity on insulin responsiveness. At 2 months of age, WT and *Alms1*^{foz/foz} males had a similar average body weight of ~24g (Fig. 1D). ITT in these mice showed that nevertheless the young nonobese *Alms1*^{foz/foz} males already exhibited significantly reduced
30 insulin responsiveness (Fig. 1E), consistent with insulin resistance preceding obesity in this model. Immunodetection of IRAP, Akt, p-AKT, GLUT4, C/EBP-α and GAPDH performed on insulin sensitive tissues namely, heart, liver, skeletal muscles and white adipose tissue (WAT) of 6-month-old non-fasted *Alms1*^{foz/foz} and WT showed no major differences in protein levels except for a consistent increase in the p-AKT to total AKT ratio in WAT, consistent with a paradoxical

increase rather than reduction in activation of upstream members of the insulin signaling pathway in glucose intolerant *Alms1^{foz/foz}* mice (Fig. 1G). To identify which tissues alone or together might be the primary source of the insulin resistance observed in *Alms1^{foz/foz}* mice, the tissue distribution of insulin-mediated deoxyglucose (DOG) uptake was compared in WT and

5 *Alms1^{foz/foz}* mice. This confirmed that severely impaired DOG uptake was limited to the WAT of *Alms1^{foz/foz}* mice with a compensatory increase in DOG uptake into the insulin-responsive gastrocnemius and soleus muscles when compared to WT mice.

These studies demonstrate that although *Alms1^{foz/foz}* mice become obese and develop progressive T2DM with age, the major initial defect contributing to insulin resistance and hyperglycaemia is

10 a failure in the absence of functional ALMS1 of adipose tissue glucose uptake in response to insulin signaling, with this defect predating the development of obesity.

EXAMPLE 2

Silencing of *Alms1* in human adipocytes blocks glucose uptake through impaired GLUT4 cellular sorting

15

Materials. From Molecular Probes, Invitrogen: Acridine Orange, Image-iT ® LIVE Plasma Membrane and Nuclear Staining Labeling Kit, 2-NBDG (2-(N-7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose), Hoechst 33258 and Cell Light TM Early Endosomes-RFP* BacMam 2.0*; Catalog #: A3568, I34406, N13195, H3569 and C10587. From Lonza: AdipoRed TM Assay Reagent (Catalog #: PT-7009). Lentiviral particles from Santa Cruz Biotechnology, INC.: ALMS1 shRNA (h) Lentiviral Particles, TBC1D4 shRNA (h) Lentiviral Particles and Control shRNA Lentiviral Particles-A; Catalog # : sc-72345-V, sc-61654-V and sc-108080 respectively. From Tocris Biosciences: Nigericin Sodium Salt (Catalog #: 4312).

20

Biochemical tests. Mice were tested for insulin resistance by the insulin tolerance test (ITT) and intraperitoneal glucose tolerance test (IPGTT). For the ITT, mice were fasted 4 hours with no

25 access to food but free access to water. Mice were weighed and insulin (Humulin R, Eli Lilly, USA) was injected ip at 0.75 U/kg body weight in 0.9% saline for injection (Pfizer, USA). Tail blood was obtained and the plasma glucose was determined for each mouse using a glucometer (Optium Xceed, Abbott, USA) and blood glucose test strips (Optium point of care, Abbott, USA)

30 at 0, 15, 30 and 60 min after insulin injection. For the IPGTT, mice were fasted 18 hours and injected at 2mg/g body weight with D-glucose (Analar, VWR, USA) in 0.9% saline for injection. Plasma glucose was determined for each mouse using a glucometer with sampling via tail vein at 0, 15, 30, 60 and 120 min after glucose injection. For plasma insulin measurement, blood was collected on conscious animals via cheek bleeding. After collection, blood samples were kept on

ice and spun at 17000g, 10min at 4°C. Insulin levels were assayed using a commercial ultrasensitive mouse insulin ELISA kit (Crystal Chem Inc., USA). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using individual mouse fasting insulin and fasting glucose levels. The following formula was used: HOMA-IR = [fasting
5 glucose (mg/dL) × fasting insulin (μU/mL)]/405.

Cell culture. Human white visceral preadipocytes (Catalog #: C-12732; PromoCell) and human mesenchymal stem cells (Catalog #: C-12974; PromoCell) derived from healthy bone marrow were purchased. The preadipocytes were seeded according to manufacturer's protocol and cultured in the Preadipocyte growth medium (Catalog #: C-27410; PromoCell) to confluence.

10 One day before inducing terminal adipogenesis, the cells were infected with specific lentiviral particles consisted of a pool of 3 shRNAs target-specific constructs purchased from Santa Cruz Biotechnology and on the next day, adipogenic differentiation was induced by changing the medium to the Preadipocyte Differentiation Medium (Catalog #: C-27436; PromoCell) for 2 days. After the differentiation phase, the medium was finally changed to the Adipocyte Nutrition
15 medium (Catalog #: C-27438; PromoCell). For the culture without insulin, Adipocyte Basal Medium (Catalog #: C-2431; PromoCell) without insulin was complemented with 5g/L of deoxyglucose, 8μg/mL d-Biotin, 400ng/mL Dexamethasone. For the hMSCs, they were cultured in Mesenchymal Stem Cell Growth Medium (Catalog #: C-28010; PromoCell) to confluence. hMSCs were transfected with specific siRNAs as described above and on the next day
20 adipogenic differentiation was induced by changing the medium to the MSC Adipogenic Differentiation Medium (Catalog #: C28011; Promocell).

RNA extraction, cDNA synthesis, q-PCR and Taqman. Total RNA was prepared from the different tissues and cells using a RiboPure™ kit (Catalog #: AM1924; Ambion) followed by a DNase treatment with the TURBO DNA-free™ (Catalog #: AM 1907; Ambion). RNA integrity
25 was assessed by gel electrophoresis and RNA concentration by Eppendorf Biophotometer Plus with the Hellma® Tray Cell (Catalog #: 105.810-uvs; Hellma). Reverse transcription of 1 μg total RNA to cDNA was performed using the BioRad iScript™ cDNA synthesis kit (Catalog #: 170-8891; BioRad). Real-time quantitative polymerase chain reaction amplification was performed in a BioRad CFX96™ Real-Time System using the iQ™ SYBR® Green Supermix
30 (Catalog #: 170-8886; BioRad) and primer sets optimized for tested targets for SYBR Green-based real-time PCR for the real-time PCR. Taqman analysis was carried out with the specific gene assay with the Taqman® Fast Advanced Master Mix (Catalog #: 4444557; Applied Biosystems). The normalized fold expression of the target gene was calculated using the

comparative cycle threshold (C_t) method by normalizing target mRNA C_t to those for GAPDH using the CFX Manager Software Version 1.5 and was verified using the Lin-Reg program.

Western blots and immunofluorescence microscopy. Male *Alms1^{foz/foz}* and WT littermates were anaesthetized. The following insulin sensitive tissues: liver, heart, muscle and adipose tissue were harvested and directly placed in RIPA buffer (Tris 50mM, NaCl 150 mM, 0.1% SDS, 1% Triton-X100) supplemented with Complete mini protease inhibitor cocktail and PhosSTOP phosphatase inhibitor cocktail (Roche, Switzerland). Samples were sonicated and centrifuged 30 min at 17 000g, 4°C 30min. Protein concentration assayed with BCA assay (Thermo Fisher Scientific, USA). Cellular proteins from cells were obtained by trichloroacetic acid precipitation and immunoblot analyses were performed using 30–50 µg total protein. Specific antibody binding was visualized using the SuperSignal ® West Femto Maximum Sensitivity Substrate (catalog #: Lf145954, Pierce) on a BioRad Versadoc TM Imaging System or ImageQuant LAS 4000 imager (GE Healthcare, UK). Nonspecific proteins stained with Ponceau S were used as loading controls to normalize the signal obtained after specific immunodetection of the protein of interest using the Bio-Rad Quantity One program. For immunofluorescence experiments, the cells were seeded on permanox 8-wells Lab-Tek II Chamber Slide (Catalog #: 177445; NUNC). Cells were treated as indicated. Then both cells and tissues cryosections were processed for protein detection after methanol fixation and permeabilized with 0.1% Triton X-100. The microscopy slides were mounted for detection with Vectashield Mounting Medium (Catalog #: H-1200; Vector Laboratories). To view membrane-associated proteins, cells were formalin fixated for 15 min and were directly blocked, followed by immunostaining and acquisition using an upright Zeiss AxioImager Z2 microscope. Image analysis, 3D reconstitution and Time Lapse experiments and endosomes tracking experiments were performed using either the Zeiss AxioVision program with the corresponding 3D and Tracking Zeiss modules or the Zeiss Zen 2012 imaging platform.

Fluorescence measurement. The preadipocytes were cultured in a 96 well plate and 12 wells infected with the either ALMS1 shRNA lentiviral particles or CTRL shRNA lentiviral particles and differentiated the next day into mature adipocytes. 3 weeks later, the intracellular triglycerides were stained with AdipoRed staining following the manufacturer's procedure and the fluorescence was measured on a Tecan Infinite 200 quad4 monochromator (Tecan, Lyon, France) at a wavelength of 520nm. The generated data were then analyzed using the Tecan Magellan Data Analysis software using as blank unstained adipocytes.

Co-Immunoprecipitation experiments. For the co-immunoprecipitation experiments, we used the Dynabeads ® Antibody Coupling kit (Catalog #: 143.11D, Invitrogen) in combination with

the Dynabeads® co-immunoprecipitation kit (Catalog #: 143.21D, Invitrogen). The hMSCs were cultured to confluence and adipogenic differentiation was triggered by medium change. 7 days after adipogenic differentiation was initiated by medium change, the adipocytes, cultured with or without Ins. 24 hours prior to lysis, were lysed under native conditions and used according to the kit. After immunoprecipitation and release from the beads, the samples were loaded on a NuPage 3-8% TrisAcetate Gel (Catalog #: EA0375BOX, Invitrogen) with a Hi Mark™ Prestained HMW Protein Standard (Catalog #: LC5699, Invitrogen).

Protein preparation and identification by mass spectrometry. In gel digestion: The gel digestion procedure was carried out as described by Rabilloud et al. (ref). Preparation of the gel pieces before trypsin digestion was performed by a liquid handler robot (QuadZ215, Gilson International, France). Briefly, gel bands were washed alternately with 100 µl of 25 mM NH₄HCO₃ and then 100 µl of 50% acetonitrile (ACN) (3 min wash under shaking and the liquid was discarded before addition of the next solvent). This hydrating/dehydrating cycle was repeated twice and the pieces of gel were dried for 20 min before reduction (10 mM DTT / 25 mM NH₄HCO₃ buffer at 56°C for 45 min) and alkylation (25 mM iodoacetamide / 25 mM NH₄HCO₃ buffer for 45 min, room temperature). Afterwards, gel spots were again washed with 3 cycles of 25 mM NH₄HCO₃ / ACN alternately. Following 20 min drying step, the gel pieces were rehydrated by three volumes of trypsin (Promega, V5111), 12.5 ng/µl / in 25 mM NH₄HCO₃ buffer (freshly diluted) and incubated overnight at room temperature. Tryptic peptides were extracted from gel by vigorous shaking for 30 min in adapted volume of 35% H₂O/60% ACN/5% HCOOH and a 15 min sonication step.

MALDI-TOF (/TOF) mass spectrometry and database search. MALDI mass measurement was carried out on an Autoflex III Smartbeam (Bruker-Daltonik GmbH, Bremen, Germany) matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF TOF) used in reflector positive mode. A prespotted anchorchip target (PAC system from Bruker Daltonik, technical note TN011) with HCCA matrix was used to analyse tryptic digests. The resulting peptide mass fingerprinting data (PMF) and peptide fragment fingerprinting data (PFF) were combined by Biotoools 3.2 software (Bruker Daltonik) and transferred to an intranet version of the search engine MASCOT (Matrix Science, London, UK). Variable modifications (N-term protein acetylation, methionine oxidation and cysteine carbamidomethylation) and one tryptic missed cleavage were taken into account and the peptide mass error was limited to 50 ppm. Proteins were identified by searching data against a NCBI non-redundant protein sequence database and then submit to the human restricted database. In all results, the probability scores were greater than the score fixed as significant with a p-value of 0.05. *NanoLC-MSMS mass*

spectrometry and database search: For nanoLC-MS/MS analysis, peptides were transferred in glass inserts, compatible with the LC autosampler system (nanoLC-U3000, Dionex, US). The LC system was coupled to an ESI-Q-TOF mass spectrometer (MicroTOFQ-II, Bruker, Germany). The method consisted in a 60 min run at a flow rate of 300 nL/min using a gradient from two solvents: A (99.9% water: 0.1% formic acid) and B (99.92% acetonitrile: 0.08% formic acid). The system includes: a 300µm X 5 mm PepMap C18 used for peptides preconcentration and a 75 µm X 150mm C18 column used for peptides elution. The TOF analyzer was calibrated each day: data were acquired and processed automatically using Hystar 2.8 and DataAnalysis 2.6 softwares. Consecutive searches against the NCBI database first and then against the human sub-database were performed for each sample using local versions of Mascot 2.2 (MatrixScience, UK) and Proteinscape 2.0 (Bruker, Germany). False-positive rate (FPR) for protein identification was estimated using a reverse decoy database: protein validation was done using a FPR below 1%. Moreover, proteins identified by only 1 peptide were checked manually: MS/MS spectra were inspected according to conventional fragmentation rules.

In situ Proximity ligation assay (PLA). Duolink in situ PLA kit with antimouse PLUS probe and anti-rabbit MINUS probe (catalog #: 90701 and 90602; OLINK Bioscience) were used in combination with the appropriate primary antibodies according to the manufacturer's procedure. Human primary preadipocytes and mature adipocytes were cultured on 8-well Lab-Tek II chamber slide (Nunc) and treated as for immunofluorescence microscopy until the primary antibody incubation step. After washing, cells were decorated with PLA PLUS and MINUS probes (1:20 dilution) for 2 hrs at 37°C. Hybridization and ligation of probes, amplification, and final SSC washing were performed according to the manufacturer's procedure. Fluorescence transfer based on protein-protein interaction was visualized using the Duolink Detection kit 613 (OLINK Bioscience) and images were acquired.

Statistics. Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc., USA). Results are shown as means ± standard deviation. Significance of the results was determined by paired *t* tests or the non-parametric Mann-Whitney U test was used for statistical comparison of BMI and AUC data. A value of $P < 0.05$ was considered to denote statistical significance and was marked with an asterisk.

Using primary human preadipocytes as an *in vitro* model, the inventors localized ALMS1 primarily in a cytoplasmic rather than the previously reported centrosomal pool. ALMS1 was silenced during adipogenesis and although a significant decrease in the anti-adipogenic factor

Pref-1 was observed, no major differences could be detected in expression levels of pro-adipogenic transcription factors such as the cEBPs and PPAR γ .

Following ALMS1 silencing in 2-week-old mature adipocytes, glucose absorption capacity was assessed using labelled glucose analogue (2-NBDG). In the absence of insulin stimulation, no 2-

5 NBDG uptake could be detected in ALMS1-silenced and control mature adipocytes (Fig. 2A).

On the other hand, insulin stimulation resulted in increased 2-NBDG uptake in the control human mature adipocytes (Fig. 2B, top panel) but not ALMS1-silenced cells (Fig. 2B, bottom

panel). Further to reduced glucose absorption in ALMS1-silenced adipocytes, the inventors observed a reduction in intracellular triglycerides (TG) in these cells a week later (Fig. 2C-D). Of

10 note, this reduced glucose absorption in ALMS1-deficient adipocytes was not associated with decreased phosphorylation of AKT, the downstream signaling target of insulin, as pS473-AKT

levels after 30 minutes incubation with insulin were similar in both control and ALMS1-silenced human adipocytes (Fig. 2E-F), consistent with the normal to increased levels of AKT

phosphorylation previously observed in *Alms1*^{foz/foz} murine adipocytes (Fig 1G).

15 The inventors next investigated the dynamics of GLUT4 in human adipocytes in the absence of ALMS1. Insulin receptor (IR) cellular localization to the plasma membrane was not impaired

following ALMS1 silencing being detected in the vicinity of the plasma membrane (PM) in the absence of insulin. (Fig. 2G, top panel). By contrast, in ALMS1-deficient adipocytes in the

20 absence of insulin GLUT4 lost its perinuclear localization and was detected dispersed throughout the cell cytoplasm rather than assuming its usual perinuclear localisation. (Fig 2G, middle and

bottom panels and 2H). Upon insulin stimulation, GLUT4 was observed to move to the PM within the actin mesh (Fig. 2I) in both control and ALMS1-silenced adipocytes. Two hours post

insulin stimulation in the absence of insulin, GLUT4 was still detected dispersed throughout the cytoplasm of the ALMS1-silenced adipocytes whereas control adipocytes had their GLUT4

25 appropriately re-localized to the perinuclear region (Fig. 2J). As there is an equilibrium between exocytosis and endocytosis of GLUT4 vesicles to and from the PM, the inventors checked to

exclude that the impaired GLUT4 sorting in *Alms1*-silenced adipocytes was not due to defective GLUT4 endocytosis. Examination of dynamin, a key molecule in endocytosis, demonstrated no

difference in protein levels nor cellular localization following ALMS1 silencing in adipocytes.

30 Furthermore, the mean velocities of labelled endosomes were similar between ALMS1-silenced and control adipocytes, arguing against a defect in endocytosis being the cause of reduced GLUT4 presence in the PM in response to insulin signaling.

EXAMPLE 3

ALMS1 is required for TBC1D4 targeting to the PM in response to insulin signaling

To understand the molecular mechanism underlying the effect of ALMS1 inactivation on GLUT4 localisation, the inventors identified interacting partners of ALMS1 in human adipocytes. Immunoprecipitation (IP) using ALMS1 as the bait was performed using young
5 mature human adipocytes (4 days after differentiation trigger) followed by identification of ALMS1 interacting partners by mass spectrometry. Amongst proteins were immunoprecipitated with ALMS1, was TBC1D4, a known AKT substrate GTPase required for proper retention of GLUT4 in the GLUT4 sorting vesicles (GSVs) and for the translocation of GLUT4 to the cell membrane for intracellular glucose uptake.

10

EXAMPLE 4**Development of Structural Homology Models of ALMS1, TBC1D4 and α PKC.**

As the crystal structure of Alms1 has not yet been solved, *in silico* structural homology modeling was used to predict the 3D structure of ALMS1 and identify structural motifs that could bind
15 potential interacting ligands (Fig. 3A-C).

Structural Model of ALMS1. The model of ALMS1 was constructed using fragment modeling method with the homology modelling program, Modeller. The amino acid sequence for each exon of ALMS1 was submitted to profile-based threading algorithm available at PISRED server and suitable templates were identified. Then those identified template proteins were aligned with
20 the respective exon sequences and each exon was modeled separately using Modeller. The energy optimization and selection of models were conducted based on discrete optimized protein energy score. Finally, models were assembled to construct the structure of full length ALMS1 and the full-length protein was relaxed and minimized using the molecular dynamics simulation program NAMD.

Structural Model of the PTP binding domain of TBC1D4. The PTP binding domain of TBC1D4 is located within the first 160 residues. No reliable homologues structure was identified to model the structure in between the PTP binding domain and the Rab binding domain. Crystal structure of the PTP domain of murine Disabled-1(Dab-1), 1NU2 (E-value=5.2e-17), which was identified by HMM based template search at Swiss model was used as the template for
25 constructing the PTP binding domain of TBC1D4.

30

The PTP domain of TBC1D4 interacting with ALMS-1. The macromolecular docking was performed by using the ClusPro 2.0 algorithm. Residues located in the interaction surface with ≥ 0.4 angstrom overlap were considered as interacting residues. Interproscan revealed that the ALMS-1 contained a WD40-like domain within the first 3871 residues. WD40 domain

containing proteins are a family of proteins functioning as scaffolds for macro-molecular interactions.

The PTP binding domain of TBC1D4 interacting with ALMS1. Initially, the PTP binding domain and the RabGTP binding domain of TBC1D4 were docked to the ALMS1 model using the Cluspro 2 server to determine the most probable site of interaction. Then both domains were docked to their respective interacting sites on ALMS1 using Autodock 4.2 and their binding affinities were calculated. Based on the affinities, the PTP binding domain of TBC1D4 binds ALMS1 with ~100 fold higher affinity compared to the RabGTP binding domain. Hence, the inventors predict that the PTP binding domain may have a higher probability to interact with the ALMS1 molecule compared to the RabGTP binding domain.

Modelling the PTP domain of TBC1D4. The phospho-tyrosine binding domain of TBC1D4 was modeled after identifying a suitable template from the Swiss model template identification algorithm.

Docking TBC1D4 PTP domain and RabGTP binding domain to ALMS1. Initially, the PTP binding domain and the RabGTP binding domain of TBC1D4 were docked to ALMS1 using the Cluspro 2 server and the site of interaction was identified. Then both domains were docked to their respective interacting sites using Autodock 4.2 and their binding affinities were calculated.

Predicted ALMS-1 residue numbers, with the potential to interact with another ligand	65,66,69,72,73,74,75,76,77,78,80,87,2875,2876,2877,2878,2879,2880,2881,2882,2883,2884,2885,2887,2888,2889,2890,2892,2893,2894,2895,2897,2909,2910,2912,2929,2931,2932,2933,2934,2935,3557,3558,4131,144,145,146,147,148,149,150,151,193,194,195,198,199,200,201,205,208,211,214,226,227,229,233,234,235,236,239,242,243,246,248,249,250,251,252,314,319,321,986,1341,1344,2269,113,114,115,116,123,126,127,128,1340,1438,1439,1440,1441,1442,1443,1444,1446,1447,1448,1449,1450,1451,1452,1453,1454,1457,1458,1459,1478,1915,1918,1919,1920,1922,1923,1930,2041,2042,2043,2257,2267,2483,2484,3866,218,219,220,221,222,223,224,277,278,279,282,285,286,287,288,686,688,689,690,691,699,1856,1858,1859,1861,1862,1863,1864,1865,1866,1867,1868,1869,1870,1871,1872,1949,1968,1969,1971,1974,1979,1980,1981,1982,1983,1984,2104,2107,2111,2870,2872,2874,2915,3285,3286,3287,793,795,796,797,1285,1314,1408,1409,1422,1423,1425,1426,1427,1430,1431,1671,1672,1794,1797,2538,2539,2540,2555,2556,2557,2563,2564,2565,2567,2568,2588,2591,2599,2603,2699,2701,2702,3108
Predicted residues from ALMS1 mediating the interaction with aPKC	E17, D58, S59, G62, H65, L66, Q736, T737, E738, D828, S829, T1088, D1089, A1169, Q1170, F2882, L2883, E2884
Predicted residues from aPKC mediating the interaction with ALMS1	F114, D116, C118, L121, N138, Q142, I145, P148, G433, E545, S562, S566, F597, D601, W602, K604, E606, G620, T631, V664, I667
Predicted residues from TBC1D4 mediating the interaction with ALMS1	G75, A76, P77, A78, R80, E81, V82, I83
Predicted residues from ALMS1 mediating the interaction with TBC1D4	H65, L66, S2879

The homology model revealed that Alms1 assumes an apple core type structure with a large number of bindings sites of potential ligands centered around the core. The TBC1D4 crystal structure was similarly not solved and hence the inventors used a homology modeling approach to predict the structure of the PTP binding domain of TBC1D4 (Fig. 3C-D). Subsequently, *in silico* docking studies were performed which predicted high affinity binding of TBC1D4 with ALMS1 through hydrogen bonding of TBC1D4 residues G75, A76, P77, A78, R80, E81, V82, I83 with interacting residues H65, L66, S2879 on Alms1 (Fig. 4A). Co-localization of ALMS1 and TBC1D4 was then confirmed in human adipocytes by immunofluorescence studies (Fig. 4B). The expression levels of GLUT4, TBC1D4, and IRAP were next tested in ALMS1-silenced adipocyte with or without insulin stimulation but no significant differences were found (Fig. 4C). Upon phosphorylation by activated AKT, phosphorylated TBC1D4 (p-TBC1D4) in adipocytes targets RAB proteins such as RAB14 and RAB10 prior to GSVs being targeted to the PM. However, upon insulin stimulation of Alms1-silenced adipocytes, no difference could be detected in the levels of TBC1D4, p-TBC1D4, RAB14 and RAB10 (Fig. 4D). The inventors next focused on GLUT4 cellular localization. In the absence of insulin stimulation, TBC1D4 silencing reproduced the ALMS1 silencing effect seen in mature adipocytes, i.e. a mislocalization of GLUT4 throughout the cytoplasm (Fig. 4E). In response to insulin stimulation, GLUT4 was released from the perinuclear region in control adipocytes spreading-out throughout the adipocyte cytoplasm (Fig. 4F) thereby reproducing the GLUT4 distribution pattern seen in in ALMS1 and TBC1D4-silenced adipocytes in the absence (Fig 4E) and presence (Fig 4F), of insulin. The inventors subsequently investigated the effect of ALMS1 silencing on the cellular dynamics of TBC1D4 in response to insulin. In the absence of insulin, TBC1D4 was localized to the perinuclear region in both control and ALMS1-silenced adipocytes (Fig. 4G) but notably, in response to insulin, TBC1D4 was only transported to the PM in control but not ALMS1-silenced adipocytes (Fig. 4H).

EXAMPLE 5

ALMS1 forms a dynamic protein complex, the *ALMSome*, required for insulin-stimulated glucose transport in human mature adipocytes

Although the inventors showed that ALMS1 silencing prevented TBC1D4 targeting to the PM, it remained to be seen whether this impairment on its own explained the major reduction in glucose uptake observed in ALMS1-deficient adipocytes. The inventors therefore compared the cellular uptake of 2-NBDG upon insulin stimulation in ALMS1 or TBC1D4-silenced or control

adipocytes and found almost no 2-NBDG absorbed in the ALMS1-silenced adipocytes compared to control adipocytes (Fig. 5A-B), whereas whilst reduced compared to controls a substantial amount of 2-NBDG was still absorbed by TBC1D4-silenced adipocytes (Fig. 5C). Subsequent GLUT4-antibody binding assays on either ALMS1 or TBC1D4-silenced or control adipocytes following 30 minutes insulin stimulation showed a high proportion of GLUT4 in the PM in control and TBC1D4-silenced adipocytes but not in ALMS1-silenced cells (Fig. 5D-F), indicating that the secondary defect in TBC1D4 targeting to the PM in *Alms1*-silenced cells did not, in itself, explain the very severe defect in glucose transport and GLUT4 PM expression in ALMS1-deficient cells. A further examination of the *Alms1* IP data revealed several subunits of V type ATPase proton (H^+) pumps (A, B, D1 and G2) that the inventors then showed to be expressed in mature adipocytes (Fig. 5G) together with α PKC, the activating kinase of the H^+ pumps under insulin control. The inventors confirmed that ALMS1 was in close vicinity with the V-ATPase H^+ pumps in mature adipocytes in the presence of insulin both by an *in situ* PLA Duolink approach targeting ALMS1 and the proton pumps subunits A1 and D1 (Fig. 5H) and also by co-immunostaining ALMS1, VATPase A1 and D1 and α PKC (Fig. 5H, I). ALMS1 co-localized with the proton pump subunit V0D1 (Fig. 5I) that is integrated into the GSV membrane indicating that ALMS1 is transported in the adipocyte together with the proton pumps localized within the GSVs. Using their *in silico*-based structural model of ALMS1 interacting partners the inventors identified a binding motif for PKC on *Alms1*. The binding sites for TBC1D4 and α PKC on *Alms1* were in such close proximity that the model showed that simultaneous docking of both proteins to *Alms1* was not possible due to steric hindrance (Fig. 5J). The inventors thus hypothesized. To test their hypothesis that ALMS1 binding of α PKC or alternatively TBC1D4 was under the reciprocal control of insulin signaling in the adipocytes the inventors performed further IPs again using ALMS1 as bait but this time using human mature adipocytes cultured in the presence or absence of insulin with IPs being immunoblotted for both α PKC and TBC1D4. The results revealed that α PKC could only be pulled down by *Alms1* and detected by immunoblotting in extracts of adipocytes incubated in the absence of insulin (Fig. 5K) whereas TBC1D4 was only pulled down by ALMS1 and detected in extracts of adipocytes incubated in the presence of insulin, consistent with the inventors model of reciprocal insulin-regulated *Alms1* binding (Fig. 5L).

EXAMPLE 6***The ALMSome is required for the acidification of GSVs prior to GLUT4 delivery to the plasma membrane***

While AKT-phosphorylation of TBC1D4 has been known to in some way lead to GLUT4
5 trafficking, the ultimate GSV-PM fusion step is an insulin regulated non-AKT dependent event
that requires osmotic swelling of the GSVs under the action of the vATPase H⁺ pump. However,
knowledge of the actual signal and mechanism for activation of the H⁺ pump by insulin was
missing. The inventors tested if ALMS1 inactivation could prevent the acidification of the GSVs
and therefore the chemo-osmotic-mediated release of GLUT4 to the PM using the acidotropic
10 dye, acridine orange, which emits a green fluorescence at low concentration and an orange-red
fluorescence at high concentrations in the lysosomes in which acridine orange is protonated and
sequestered. In absence of insulin, no orange-red fluorescence was detected in the adipocytes. By
contrast, insulin induced a rapid appearance of red color in control human mature adipocytes
(Fig. 6A) but not in ALMS1-silenced adipocytes (Fig. 6B) indicating loss of insulin-mediated
15 acidification of lysosomes in ALMS1-silenced adipocytes.

The inventors next tested whether acidifying ALMS1-silenced adipocytes using Nigericin
(NIG.), an electroneutral K⁺/H⁺ exchange ionophore known to cause osmotic swelling of the
GSVs would bypass the Alms1-associated defect in GLUT4 fusion and glucose absorption. NIG.
treatment resulted in a rapid acidification of both control and ALMS1-silenced adipocytes (Fig.
20 6C-D), thereby activating the swelling and fusion of the intracellular vesicles. In parallel,
electron microscopy analysis showed vesicles sitting next to the PM without fusion in absence of
insulin in both control and ALMS1-silenced adipocytes (Fig. 6E-F, top panels). Insulin treatment
caused a swelling of the vesicles (Fig. 6E, middle panels) associated with fusion of vesicles with
the PM for glucose absorption only in the control adipocytes (Fig. 6E, middle panels) but not in
25 ALMS1-silenced adipocytes (Fig. 6F, middle panels). However, NIG. induced vesicular swelling
and fusion with the PM in both control and ALMS1-silenced adipocytes (Fig. 6E-F, bottom
panels). The NIG treatment restored glucose absorption in ALMS1-silenced adipocytes. While
insulin had little effect in inducing 2-NBDG absorption in ALMS1-silenced adipocytes (Fig. 6G-
H, top and middle panels), NIG not only restored vesicle fusion but could also be shown to
30 restore 2-NBDG absorption in the ALMS1-silenced adipocytes to levels seen in control cells
(Fig. 6G-H, bottom panels) This restored glucose transport in NIG-treated ALMS1-silenced
adipocytes correlated with restored GLUT4 fusion with the PM (Fig. 7A) but not with TBC1D4
targeting to the PM (Fig. 7B); and led to restoration of TG-filling of ALMS1-silenced adipocytes
24-hours post NIG treatment.

EXAMPLE 7**Identification of peptide inhibitors of PKC binding to Alms1**

Once the site of binding interaction between two proteins is known, as known in the art it is possible using knowledge of the conformation and amino acids of each protein involved in mediating the interaction, to use computational models to design peptides or small molecule drugs which by binding in the region of the interaction site are able to sterically or otherwise hinder the binding interaction. The inventors therefore sought to identify peptides that would inhibit the interaction of ALMS1 and α PKC or TBC1D4 using their previously described ALMS1, TBC1D4 and α PKC structural models described in Example 4. Peptides predicted using this method to block the interaction between α PKC and ALMS1 included the sequences LDSDSHYGPQHLESIDD (SEQ ID No 5), DSHQTEETL (SEQ ID No 6), QQTLPESHLP (SEQ ID No 7), QALLDSHLPE (SEQ ID No 8), PADQMTDTP (SEQ ID No 9), HIPEEAQKVSAV (SEQ ID No 10) or SCIFLEQ (SEQ ID No 11). A peptide identified using this method to block the interaction between TBC1D4 and ALMS1 was the sequence GCGAPAAREVILVL (SEQ ID No 12).

EXAMPLE 8**Expression of the specific ALMS1-interacting α PKC interacting domain in mature adipocytes triggers glucose absorption in absence of insulin.**

Next, the inventors verified the hypothesis that insulin mediates the release of α PKC from the ALMSome complex in order to induce glucose absorption. For that, they cloned the interacting domain of α PKC (SEQ ID Nos 14 and 15) in a lentiviral vector together with a Flag-TAG. The selected sequence was the minimum sequence of α PKC (min- α PKC-FLAG) so as to prevent sterical hindrance with the TBC1D4 interaction site on ALMS1. The expressed min- α PKC-FLAG in the adipocytes competes with the endogenous α PKC to prevent it from binding Almsome and hence favor the insulin-mediated TBC1D4 binding to Almsome. Mature adipocytes were then infected with either control or min- α PKC lentiviral particles to assess the impact of min- α PKC-FLAG on glucose absorption. 48 hours post-infection, min- α PKC-FLAG was immunodetected using an antibody against the FLAG-Tag (Fig .9). For the *in vitro* proof of concept, we treated mature adipocytes as described (Fig. 9) and then incubated the treated mature adipocytes with 2-NBDG to assess the effect of min- α PKC-FLAG on glucose absorption. Of interest, 2-NBDG was absorbed in min- α PKC-FLAG treated adipocytes in absence of INS (Fig.8A, left column) which corresponded to a 3.5 times increase compare to control (Fig. 8B).

On the other hand, no significant difference was observed in presence of INS (Fig. 8A, right column and 8B). These data demonstrate that targeting the interaction of ALMS1 and α PKC is sufficient to trigger glucose absorption in the adipocytes irrespective of the presence of INS.

Production of lentiviral vector carrying the α PKC domain

5 The ALMS1-interacting domain of human PKC α was amplified from human HEK293 cell cDNA with N-terminal FLAG tag using Forward 5'-gtacGAATTCGCCACCATGGATTACAAGGATGACGACGATAAGCTCACGGACTTCAAT TTCCTC -3' (SEQ ID No 16) and Reverse 5'-tagcGGATCCTCATACTGCACTCTGTAAGATGGG-3' (SEQ ID No 17) primers and cloned
10 into lentiviral vector pCDH-EF1-MCS-IRES-puro (System Biosciences). For virus production, PKC α lentiviral vectors were transfected into 293TN cells (System Biosciences) along with packaging plasmids psPAX2 and pMD2.G (Addgene) with the weight ratio of 3:2:1 respectively by using Lipofectamine 2000 (Life Technologies). Forty-eight hours after transfection, the culture supernatant was harvested by centrifugation at 500 $\times$ g for 10min, followed by filtration
15 through 0.45 μ m syringe filter with PES membrane (Sartorius). The virus solution was then concentrated by adding 1/2 volume of cold 30% (wt/vol) PEG6000 dissolved in 0.5M NaCl and incubated overnight at 4°C with occasional mixing. The mixture was then centrifuged at 3000 $\times$ g for 15min at 4°C. Then the pellet containing lentiviral particles was resuspended in 1mL DMEM medium and stored at -80°C before infection of target cells.

CLAIMS

- 1- A molecule preventing the binding of α PKC (Protein Kinase C alpha type) to ALMS1 (Alstrom syndrome protein 1) for use in treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia.
- 2- The molecule for use according to claim 1, wherein the molecule is for use in treating or delaying the progression or onset of type 2 diabetes mellitus.
- 3- The molecule for use according to claim 1 or 2, wherein the molecule does not interfere with the binding of TBC1D4 to ALMS1.
- 4- The molecule for use according to anyone of claims 1-3, wherein the molecule is selected from the group consisting of peptides or polypeptides or peptide mimetics, antibodies, fragments or derivatives thereof, aptamers, Spiegelmers, and chemical compounds.
- 5- The molecule for use according to anyone of claims 1-4, wherein the molecule is a peptide less than 50 amino acids, preferably less than 20 amino acids.
- 6- The molecule for use according to claim 5, wherein the molecule is a peptide comprising an amino acid sequence of a fragment of ALMS1 (SEQ ID No 1).
- 7- The molecule for use according to claim 6, wherein the molecule is a peptide comprising an amino acid sequence of a fragment of ALMS1 including one or several of the residues which are predicted to mediate the interaction with α PKC, in particular one or several of the residues selected in the list consisting of E17, D58, S59, G62, H65, L66, Q736, T737, E738, D828, S829, T1088, D1089, A1169, Q1170, F2882, L2883, and E2884.
- 8- The molecule for use according to claim 6 or 7, wherein the molecule is a peptide comprising or consisting of one of the following sequences:
 - LDSDSHYGPQHLESIDD (SEQ ID No 5);
 - DSHQTEETL (SEQ ID No 6);
 - QQTLPESHLP (SEQ ID No 7);
 - QALLDSHLPE (SEQ ID No 8);
 - PADQMTDTP (SEQ ID No 9);
 - HIPEEAQKVSAV (SEQ ID No 10);
 - SCIFLEQ (SEQ ID No 11), and
 - a fragment thereof comprising 6 contiguous amino acids.
- 9- The molecule for use according to claim 5, wherein the molecule is a peptide comprising an amino acid sequence of a fragment of α PKC (SEQ ID No 4).

- 10- The molecule for use according to claim 9, wherein the molecule is a peptide comprising an amino acid sequence of a fragment of α PKC including one or several of the residues which are predicted to mediate the interaction with ALMS1, in particular one or several of the residues selected in the list consisting of F114, D116, C118, L121, N138, Q142, I145, P148, G433, E545, S562, S566, F597, D601, W602, K604, E606, G620, T631, V664, and I667.
- 11- An *in vitro* or *ex vivo* method for identifying molecules suitable for use for treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia, wherein the capacity of the molecule to prevent the binding of α PKC to ALMS1 is assayed and the molecules capable of preventing this binding are selected.
- 12- The method of claim 11, wherein the method further comprises a step in which the capacity of the selected molecule to interfere with the binding of TBC1D4 to ALMS1 is tested and wherein the molecules which do not interfere are selected.
- 13- The method of claim 11 or 12, wherein the binding is determined in a cellular system responsive to insulin.
- 14- The method of any one of claims 11 to 13, wherein the binding is determined in presence and/or absence of insulin.
- 15- A molecule as defined in any one of claims 6-8 and 10 for use as a drug.
- 16- A molecule increasing the expression of ALMS1 or enhancing the binding of TBC1D4 to ALMS1 for use in treating or delaying the progression or onset of diabetes mellitus, insulin resistance, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, insulin resistance, hyperglycemia, obesity, and hyperinsulinaemia