UCP2 INTERACTOME AS TARGETS FOR NOVEL ANTI-DIABETIC DRUGS

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ABSTRACT

Mitochondrial dysfunction contributes significantly to the pathogenesis and progression of diabetes. This study assessed the role of mitochondrial dysfunction in diabetes onset and progression, identified the proteins that interact with UCP2 and evaluated their suitability as targets for novel anti-diabetic drugs. Proteins interacting with UCP2 were predicted using STRING 9.0. Among the proteins identified as veritable targets for novel anti-diabetic drugs are leptin (LEP), peroxisome proliferator-activated receptor gamma (PPARG), ghrelin/obestatinprepropeptide (GHRL), Neuropeptide Y (NPY), peroxisome proliferator-activated receptor gamma (PPARA), Adiponectin (ADIPOQ), peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGCIA), ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (ATP50), and forkhead box A2 (FOXA2). The E values were predicted using STRING version 9.0. The predicted interactions are satisfactory and are supported by text mining, occurrence, neighborhood, database, fusion, experimental, and co-expression data. Development of lead compounds against these targets will help to address the burden of diabetes and provide more effective and safer anti-diabetic medicines.
In summary, our findings indicate that molecules that interact with UCP2 are leptin (LEP), PPARG, GHRL, Neuropeptide Y, PPARA, ADIPOQ, PPARGCIA, ATP50, and FOXA2. Validation of these targets and development of lead compounds against the targets will give rise to novel anti-diabetic drugs that will be much more effective help to address the burden of diabetes and provide more effective and safer anti-diabetic medicines.

Keywords: mitochondrial dysfunction - UCP2 - diabetes

RESUME

Le dysfonctionnement mitochondrial joue un rôle majeur dans la pathogénèse et la progression du diabète. Cette étude mets en évidence le rôle du dysfonctionnement mitochondrial dans l'installation et l'évolution du diabète, et identifie les protéines qui interagissent avec le ''UCP2'' en évaluantleur utilisationcomme les nouveaux traitements antidiabétiques. Les protéines interagissant avec ''UCP2'' ont été identifiées avec le logiciel STRING (version 9.0). L'échantillon des protéines ciblées par les nouveaux traitements antidiabétiques comprennent les molécules suivantes: ''leptin (LEP)'', ''peroxisomeproliferator-activatedreceptor gamma (PPARG)'', ''ghrelin/obestatinprepropeptide (GHRL)'', ''Neuropeptide Y (NPY)'', ''peroxisomeproliferator-activatedreceptor gamma (PPARA)'', ''Adiponectin (ADIPOQ)'', ''peroxisomeproliferator-activatedreceptor gamma'', ''coactivator 1 alpha (PPARGCIA)'', ''ATP synthase'', ''H+ transporting'', ''mitochondrial F1 complex'', ''O subunit (ATP50)'', et le ''forkhead box A2 (FOXA2)''.Les paramètres E furent établis grâce au logiciel STRING (version 9.0). Les interactions obtenues sont satisfaisantes et sont renforcées par une recherche approfondie, une détection de la survenue, une collecte des informations, une fusion et une analyse expérimentale des données actuelles. L'élaboration des techniques de protection pour ces protéines ciblées permettra d'exposerl'impact du diabète, et donnera des traitements plus surs et plus efficaces.

Mots-clés: Dysfonctionnement mitochondrial - UCP2 - Diabète

INTRODUCTION

It is known that diabetes is a chronic debilitating and non-communicable disease caused by aberrant carbohydrate utilization. The defining characteristic of diabetes is high levels of blood glucose or hyperglycemia [1]. However, Type 2 diabetes (T2DM) is the commonest form of diabetes and is defined by hyperglycemia, steady loss of β cell function, and resistance to insulin [2]. However, T2DM accounts for 90% to 95% of all diabetes cases globally [3].Type1Diabetes (T1D) is characterized by an absolute lack of insulin production due to the destruction of pancreatic β cells. This type of diabetes afflicts about 10% to 15% of all diabetic patients and is typically managed by placing the patients on lifelong injection of exogenous insulin[4]. Increasingly, a third type of diabetes referred to as mitochondrial diabetes is being recognized as a distinct subset. Mitochondrial diabetes is usually observed in middle age, is transmitted maternally, and patients usually have loss of hearing. Mitochondrial diabetes occurs due to heteroplastic mutations in mitochondrial genes. Whereas T1D is largely genetic, the aetiology of T2DM is associated with genetic factors, environmental factors, immune factors, and idiopathic factors [5]. Environmental factors include excessive energy intake and sedentary factors and these lead to overweight and obesity which are important risk factors for T2DM. Genetic factors are important aetiological causes of T2DM and a number of single nucleotide polymorphisms (SNPs) that make individuals susceptible to obesity hence increasing their risk of T2DM have been isolated [6]. More than 40 genes linked to T2DM have been identified through genome-wide association studies (GWAS) with many of these genes associated with obesity, insulin resistance, or dysfunction of β cells [7].

Recent studies however indicate that mitochondrial dysfunction is a key cause of T2DM, type 1 diabetes, and mitochondrial diabetes. The studies also suggest that diabetes complications are in large perpetuated by dysfunctional mitochondria. The mitochondrial uncoupling protein (UCP2) plays a central role in the disease onset and progression as it is activated by reactive oxygen species (ROS) generated in dysfunctional mitochondria to

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inhibit the release of ATP hence prevent translocation of the glucose transporter 4 (GLUT4) to the membrane [8]. The proposed mechanism for mitochondrial dysfunction as a cause of diabetes is described as follows. Dietary carbohydrates are digested in the lumen into simple sugars such as glucose and galactose. The glucose is then transported across the brush border membrane of the small intestines into intestinal epithelial cells by SGLT1 and GLUT2 transporters. The glucose transporter type 4 (GLUT4) in turn transports glucose from the epithelial cells and across the basolateral membrane into the bloodstream. Sequestration of GLUT4 in intracellular vesicles in adipocytes and muscle cells is a normal occurrence. Production of insulin triggers the translocation of GLUT4 to the plasma membrane from these vesicles thereby enhancing the absorption and transport of glucose [9].

The exact mechanism through which insulin enhances the availability of GLUT4 transporters on the plasma membrane has been elucidated. The insulin receptor is a transmembrane domain receptor that belongs to the tyrosine kinase family. It has 2 extracellular alpha and 2 beta transmembrane subunits which are interconnected via disulfide bonds. The alpha subunits form the insulin-binding domain while the cytoplasmic portions of the beta subunits are tyrosine kinase domains [9] .

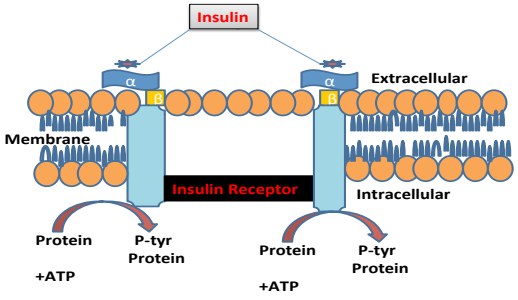

Figure 1: Insulin receptor

The first step involves the binding of insulin to its receptor. Attachment of insulin to the alpha subunits of the insulin receptor leads induces the autophosphorylation of the beta subunits. Autophopshorylation of the tyrosine kinase domain of the insulin receptor induces the recruitment of the insulin receptor substrate (IRS). The IRS attaches to the SH2 domain of the phosphatidy insoitol-3 (PI3) kinase via its pTyr domain.Phosphatidyl inositol-2 (PIP-2) is thereafter converted to PIP-3 by the PI-3 kinase. Protein kinase B is phosphorylated and this causes phosphorylation of TBC1D4, leading to the inhibition of the latter's GAP domain and hence allowing the conversion of the Rab protein from its GDP to GTP bound state. Proteins in the cascade are sequentially activated leading to the expression of GLUT4 on the plasma membrane.The GLUT4 allows facilitated diffusion of circulating glucose to occur down its concentration gradient into adipocytes and muscle cells. The glycokinase and hexokinase enzymes then phosphorylate the glucose to form to form glucose-6-phosphate (G6P). The G6P gets into the glycolytic pathway and is subjected to polymerization into glycogen [9]. Mitochondrial dysfunction is thought to lead to diabetes by inhibiting fatty acyl-CoA oxidation and by generating ROS. Mitochondrial dysfunction impedes the oxidation of fatty acyl-CoA hence leading to an accumulation of diacylglycerol (DAG) and fatty acyl-CoA and activation of protein kinase C (PKC)8. Production of ROS and deranged acyl-CoA oxidation lead to activation of serine kinases which in turn causes the phosphorylation of IRS-1 impeding the transduction of insulin signal by inhibiting the cascade that leads to Akt activation and consequent translocation of GLUT-4 vesicles [8](figure 2).

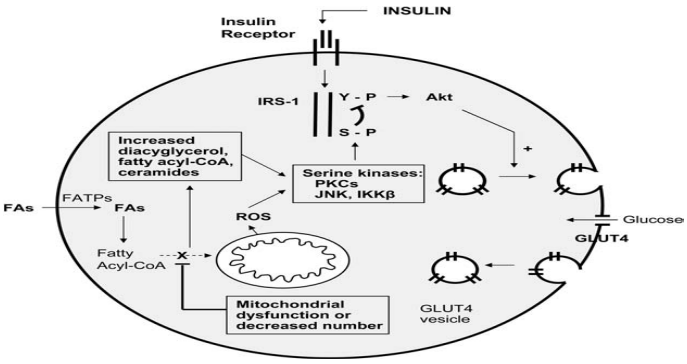

Figure 2: impaired oxidation of fatty acyl-CoA reduces glucose uptake by cells

Mitochondrial dysfunction interferes with insulin signaling by producing reactive oxygen species [8]. The ROS are thought to predispose one to diabetes as described below:

a) ROS damage the pancreatic beta cells [9-15]
b) ROS cause oxidative damage to tissues that are sensitive to insulin[16-18]
c) ROS lead to the complications associated with diabetes.

This occurs as a result of the fluctuation of fatty acids and glucose to mitochondria leading to production of ROS which damage the vasculature [19-21]. For instance, peroxynitrite is formed by the reaction between nitric oxide and superoxide. Peroxynitrite stimulates peroxidation of lipids and eats up nitric oxide leading to impairment of the vasodilation that is mediated by the endothelium. Additionally, superoxide destroys iron-sulfur centers and this makes it impossible for aconitase and other enzymes to catalyze reactions. Complications associated with ROS production arising from mitochondrial dysfunction include diabetic nephropathy [22,23] and diabetic retinopathy[8]. ROS increase the risk for cardiovascular events by about four times[8].

ATP from the TCA cycle greatly influences the sensing and release of insulin. This is because ATP is required for the potassium channels to open thereby allowing the entry of calcium ions and release of insulin from storage granules. Hyperglycemic conditions induce the formation of reactive oxygen species which induce the formation of the uncoupling protein 2 (UCP2) which causes oxidative damage, impeding the formation of ATP. Once ATP formation is impeded, potassium channels cannot open

and the entry of calcium is hindered. This hampers the release of insulin from the storage granules [8].

The role of UCP2 in diabetes is further strengthened by findings which indicate that hyperglycemic mice experience a reduction in hyperglycemia when the UCP2 gene is knocked down. The secretion of insulin is further impaired in pancreatic beta cells following over-expression of the UCP2 gene [8].

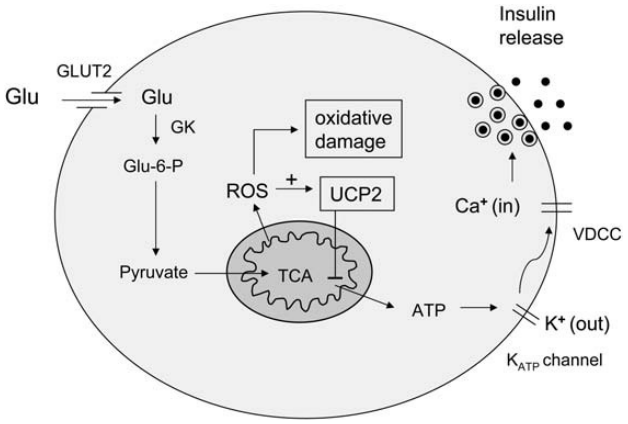

Figure 3: Effect of ROS on mitochondrial dysfunction.

ROS activate UCP2 which inhibits ATP formation hence impedes insulin release. Source: (Sivitz & Yorek, 2009)
Key: VDCC, voltage dependent calcium channel; GK, glucokinase; TCA, tricarboxylic acid cycle; ROS, reactive oxygen species; UCP2(uncoupling protein 2); GLUT2, glucose transporter 2.Thus,this paper evaluates the role of mitochondrial dysfunction in diabetes, assesses the therapeutic interventions used in the management of diabetes, and identifies and evaluates proteins in the UCP2 interactome which are putative targets for the development of novel anti-diabetic drugs.

METHODS

Network pathway tools including Biocyc (available at http://biocyc.org/) and KEGG (available at http://www.genome.jp/kegg/) were searched for networks with the ucp2 protein but no hits were found. Using NCBI's Entrez engine, the nucleotide sequence of UCP2 gene was obtained (appendix 1) and subjected to six-frame translation using the Orf Finder Tool (available at http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The longest sequence was identified as +3 on the plus strand with 931 nucelotides spanning position 381 to position 1310. BLAST was run to identify proteins with similar sequences with the default options selected. The non-redundant database was used, models and uncultured and environmental samples excluded, the program optimized for megablast, and low complexity regions filtered. The first hit for Homo sapienswith 100% query coverage, 100% identity, and an E value of 0 was selected and the Seqdump file for the protein sequence used.

The SMART database (available at http://smart.embl-heidelberg.de/) was used to assess the functional domains of the protein and to map its interactome. The normal mode was selected and the protein sequence from the seqdump file pasted. Outlier homologues, PFAM domains, signal peptides, internal repeats, and intrinsic protein disorder were checked and the Sequence SMART button pressed. The confidently predicted domains were noted and the protein-protein interactions for ucp2 identified using the STRING version 9.0 software. The proteins interacting with the ucp2 were then evaluated for their suitability as targets for diabetic drugs in humans.

RESULTS

Three protein domains were predicted confidently and these are tabulated below together with their E values.

Table 1: Confidently predicted domains

Domain	start	End	E-value
Pfam:Mito_carr	211	301	5.7e-23
Pfam:Mito_carr	112	208	4.1e-27
Pfam:Mito_carr	10	111	5.8e-24

1.1. Protein Interactions

Predictions on the proteins interacting with ucp2 were made using STRING and using neighborhood, gene fusion, co-occurrence, co-expression, homology, text mining, and experimental data and techniques (figure 5).

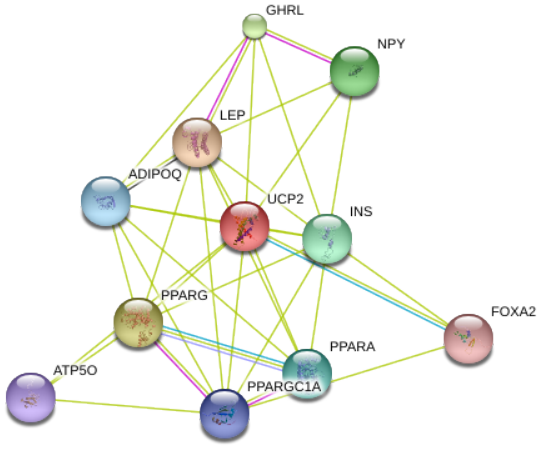

Figure 4: proteins interacting with ucp2

The proteins identified as interacting with ucp2 include Leptin (LEP), peroxisome proliferator-activated receptor gamma (PPARG), ghrelin/obestatin prepropeptide (GHRL), Neuropeptide Y (NPY), peroxisome proliferator-activated receptor gamma (PPARA), Adiponectin (ADIPOQ), peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGCIA), ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (ATP50), and forkhead box A2 (FOXA2). The predicted

scores for these interactions are tabulated alongside the proteins below.

Table 2: Predicted functional partners of UCP2 protein

Protein	Score
Leptin (LEP)	0.990
peroxisome proliferator-activated receptor gamma (PPARG)	0.990
ghrelin/obestatinprepropeptide (GHRL)	0.988
Neuropeptide Y (NPY)	0.980
Insulin (INS)	0.978
peroxisome proliferator-activated receptor gamma (PPARA)	0.968
Adiponectin (ADIPOQ)	0.964
peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGCIA)	0.960
ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (ATP50)	0.939
forkhead box A2 (FOXA2)	0.927

Prediction was based on neighborhood, gene fusion, co-occurrence, co-expression, experimental, database, and text mining data.
The phylogenetic profile is defined by the organisms containing the query sequence and those that do not. Functionally associated proteins often have similar phylogenetic profiles. The queries (COGs or proteins) are across the top of the table, and species are down the tree to the left of the table. The presence or absence of an item in an organism is marked with a green tick mark in COG-mode, or with a quantitative color scale in Protein-mode (showing the amount of sequence conservation between your protein and its best hit in the other species, dark brown being 100% identity).

DISCUSSION

As discussed above, the role of mitochondrial dysfunction in the onset and progression of diabetes is supported by a large body of evidence. Reduction in the size, cristae, and density of mitochondria in diabetic subjects is supportive of this role of mitochondrial dysfunction [8]. Also supporting the role of mitochondrial dysfunction in diabetes are the findings that diabetic subjects manifest an impairment in the biogenesis of mitochondria [8].However, reduction in proteins such as presenillin-associated rhomboid-like (PARL) mitofusin (MFN) and which are vital in mitochondrial fusion and fission, impairment of oxidative phosphorylation, inability to switch substrate utilization, and reduced mitochondrial functionality [8]. Whereas these studies prove that mitochondrial dysfunction is an essential process in the onset and progression of diabetes, very little has been done to come up with anti-diabetic medicines that selectively target proteins involved in the mitochondrial dysfunction pathway. This has worked against efforts to contain the condition. By identifying the proteins critical for mitochondrial dysfunction in diabetes, the study provides a blueprint that can be used to design novel molecules that would be more effective in containing diabetes.

Furthermore, since they do not target the mitochondrial dysfunction pathway, existing anti-diabetic drugs do not effectively stop the progressive loss of β cell function. They also do not adequately reduce body weight. Whereas intensive management of diabetes with insulin has been proven to be effective in controlling the glycaemic burden, it is costly and invasive since it usually involves injections with exogenous insulin. The drugs may also lead to hypoglycemia, may fail to adequately control body weight as well as fail to lower the levels of HbA1c and control the glycaemic burden [24, 27]. Alpha-glucosidase inhibitors are associated with flatulence and provide only a marginal decrease in HbA1c levels, pioglitazone can cause bladder cancer and heart failure when combined with insulin, and rosiglitazone is associated with the risk of cardiovascular failure. Sulfonylureas are associated with the risk of hypoglycaemia and can lead to weight gain and altered liver function leading to hepatic failure or hepatitis [28,30].

Incretin hormones have a short half life meaning that they are labile. However, new advances have helped to extend their half-lives [31]. Other drawbacks of these drugs are that they are costly and are characterized largely by inconclusive data. They are also associated with a range of adverse effects that range from GIT disturbances to headache to cancers of the thyroid and pancreas, among others (table 1) [32]. Incretin-based drugs are also largely indicated for use by patients with T2DM under metformin monotherapy and whose blood glucose levels are not sufficiently controlled and are ideal for use as an initial therapy for patients who cannot use metformin due to contraindications and target both basal and post-meal hyperglycaemia [33]. Finally, it is evident that mitochondrial dysfunction is a key event in the pathogenesis and progression of diabetes. Except for thiazolidinediones which improve the mitochondrial function of fat cells leading to insulin sensitization, many of the currently available anti-diabetic drugs do not address perturbations due to mitochondrial dysfunction.

The above considerations necessitate the development of new therapies for the management of diabetes. Identification of putative targets for drugs targeted against mitochondrial dysfunction will lay the foundation for the identification of lead compounds and their development into drugs.

Thus, using STRING 9.0, we were able to determine the proteins that interact with ucp2.The proteins include Leptin (LEP), peroxisome proliferator-activated receptor gamma (PPARG), ghrelin/obestatin prepropeptide (GHRL), Neuropeptide Y (NPY), peroxisome proliferator-activated receptor gamma (PPARA), Adiponectin (ADIPOQ),

peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGCIA), ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (ATP50), and forkhead box A2 (FOXA2). The prediction scores support the interaction and are affirmed by the different views provided in STRING. The neighborhood view usually displays genes that are connected by black lines and which are in immediate neighborhood on the genome (within 300 bp on the same strand). Genes having multiple colors map to several relevant items (orthologous groups/proteins), indicative of putative fusion events. Small white genes are neighbors, but not high-scoring enough [34, 35]. In our case however, no neighborhood events were visible. Other prediction methods are the gene fusion, occurrence, co-occurrence, experimental, text mining, and database methods. In the gene fusion display, genes mapping to multiple items are shown as multicolored units [34, 35].The occurrence view determines the organisms in which the query sequence is conserved. The rationale is that functional partners often have similar occurrence patterns. The phylogenetic profile is defined by the organisms containing the query sequence and those that do not. Functionally associated proteins often have similar phylogenetic profiles. The queries (COGs or proteins) are across the top of the table, and species are down the tree to the left of the table. The presence or absence of an item in an organism is marked with a green tick mark in COG-mode, or with a quantitative color scale in Protein-mode (showing the amount of sequence conservation between your protein and its best hit in the other species, dark brown being 100% identity) [34, 35]. With exception of the gene fusion display, all the other displays provide supporting evidence about the credibility of the interactions. For instance, the text mining display provides evidence from published journals about the interactions between ucp2 and the identified proteins. The evidence is supported by studies by Regnault et al on PPAR [36], Qian et al [37] on leptins, Sun et al [38] on ghrelin, Yonezawa et al[39] on octanoate, and 50 other authors. With regard to database evidence, the data above is supported by KEGG's adipocytokine signaling pathway and Huntington's disease pathway, and other curated pathways such as the NR-MED1 Coactivator Complex. More than 50 abstracts from PubMed also lend support to the data obtained above. However, more work needs to be done to validate these putative protein targets so that their utility in drug design can be ascertained.

Data obtained from this study also emphasizes the cardinal role of antioxidants in the prevention of the onset and progression of diabetes. The rationale behind the use of antioxidants is that prevention of ROS production will help reduce damage to beta pancreatic cells, reduce oxidative damage to both insulin-sensitive and insulin-insensitive cells, and help prevent diabetic complications. Available antioxidants include Vitamin C, Vitamin E, Coenzyme Q, Manganese, Iodide, Melatonin, Flavonoids, Carotenoid terpenoids, Capsaicin, Chicoric acid, Salicylic acid and Phenolic acid. Most of these are naturally available in vegetables and fruits and this further underlines the role of diet in the management of diabetes.

In the United States, diabetes mellitus is the number one cause of limb amputations and blindness globally [3]. T2DM is also the leading cause of end stage renal disease (ESRD) and impotence in the U.S. and Europe [3]. People who have diabetes are 4 times likelier to develop stroke and cardiovascular illnesses than those who don't have diabetes [3]. The likelihood of developing gangrene is 50 times higher in diabetics than it is in people without diabetes [3, 40]. Diabetes mellitus imposes profound economic losses to individuals and nations. For instance, $174 billion was the cost of the disease in the U.S. in 2008 alone [41]. Other factors that make T2DM a global health concern is that it is a major cause of disabilities, it causes significant loss of productivity and hospitalization, and it generally leads to a lower quality of life [3]. Validation and development of lead compounds targeting UCP2 and the identified interacting proteins is important as it will help to reduce the burden of diabetes hence reduce the high rate of mortality associated with T2DM as well as minimize complications, loss of productivity, and economic losses brought about by the disease.

CONCLUSION

In summary, our findings indicate that molecules that interact with UCP2 are leptin (LEP), PPARG, GHRL, Neuropeptide Y, PPARA, ADIPOQ, PPARGCIA, ATP50, and FOXA2. Validation of these targets and development of lead compounds against the targets will give rise to novel anti-diabetic drugs that will be much more effective help to address the burden of diabetes and provide more effective and safer anti-diabetic medicines.

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