Novel mechanisms for prevention and treatment of type 2 diabetes

Li, Naishi

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Curiosity, obsession and dogged endurance, combined with self-criticism, have brought me to my ideas.

Albert Einstein

The task is ... not so much to see what no one has yet seen; but to think what nobody has yet thought, about that which everybody sees.

Erwin Schrödinger

To my dear wife, Naijun
and our unfortunate son

Paranimfen: Tobias Wijshake
Marijke Rianne van der Sijde

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CHAPTER 1

General Introduction

1. History of T2D pharmacotherapy since discovery of insulin and current situation

1. 1 Brief history of pharmacotherapy of T2D

100 years ago, diabetes mellitus was a lethal disease, which was possibly seen as worse than cancer is considered today. There were no effective treatments to change the fate of diabetes patients, except that starvation intervention seemed to slightly delay the process to death[1].

The discovery of insulin was a milestone in the history of diabetes treatment. Since 1922, when insulin was introduced as a clinical treatment, diabetes is no longer considered a severe disease[2]. Millions of diabetes patients are able to live normal lives with insulin.

However, insulin cannot solve all the problems of diabetes patients. One problem is that a larger dosage of insulin should be used for some patients to control their plasma glucose concentrations. In 1936, Prof. Himsworth published a paper on differentiating diabetes patients into insulin-sensitive and insulin-insensitive types[3]; these are now called type 1 diabetes (T1D) and type 2 diabetes (T2D). T1D means the patient has a lack of insulin, while T2D means there is not only relatively insufficient insulin, but that it is also ineffective in utilizing the glucose efficiently; this is now called “insulin resistance (IR)”.

Since tolbutamide and metformin were introduced as therapy in the 1950s, a number of oral anti-diabetic drugs for T2D have been developed (Box 1). Each type of drug has its own mechanism of effect, and we now have several options

Box 1. Oral anti-diabetic drugs and their mechanisms

Oral anti-diabetic drugs	*Mechanism*
Sulfonylureas/glinides	Stimulates beta-cells to secret more insulin
Metformin	Inhibits hepatic glucose production
Alpha-glucosidase inhibitors	Delays absorption of carbohydrates by intestine
Pioglitazon	Enhances the activity of GLUT4
DPP-IV inhibitor	Increases $t_{1/2}$ of GLP-1
SGLT2	Increases renal glucose elimination

for treating T2D[4]. Metformin, sulfonylureas/glinides, alpha-glucosidase inhibitors, thiazolidinediones and insulin constitute the current pharmacotherapy for T2D.

1. 2 Chronic complications and difficult problems for pharmacotherapy

Since the lives of diabetes patients have been greatly extended by insulin therapy, clinical physicians now observe chronic complications more frequently, including disorders of retina, kidney, nervous system, heart, etc. These are still difficult problems for clinicians to treat, even today. Although many types of drugs (metformin, sulfonylureas, alpha-glucosidase inhibitors, thiazolidinediones, etc.) have been developed since the 1950s, the effects of anti-diabetic treatments are always disappointing to some extent. If we compare T2D with infectious diseases, we see that most of those diseases have been solved by antibiotics, whereas if we compare T2D with hypothyroidism, a fairly similar endocrine disorder with insufficient hormone production, we have to admire that those patients can live normal lives by simply taking a tablet once a day. Although we can control the blood glucose levels so that they are similar to normal and the chronic microvascular complications can be delayed, the macrovascular complications, which were implicated by UKPDS[5], do not seem to be improved. The consoling conclusion is that metformin is effective in delaying the process of chronic complications[6]. Hence, although we can do something towards preventing chronic complications, the effect is still far from satisfactory.

1. 3 New drugs with different mechanisms

It was hoped new drugs with different mechanisms would solve the problem of chronic and longer term complications. A drug delaying the process of chronic complications would be helpful for most T2D patients. Glucagon-like peptide-1 (GLP-1) receptor agonists for injection (or oral dipeptidyl peptidase-4 inhibitor, which can protect GLP-1 from hydrolysis) and type 2 sodium glucose co-transporters (SGLT2) are two new types of anti-diabetic drugs with new mechanisms (Box 1). The former is based on the function of gastrointestinal hormones, while the latter stimulates urinary excretion of glucose[7]. However, nobody knows yet whether they will be able to stop the natural progression of T2D.

1. 4 Potential drug targets from genetic research

New technology has made the genetics of diabetes a rapidly progressing field[8]. Completion of the Human Genome Project was the landmark leading to

the start of the genetic era in diabetes research. Genome-wide association studies (GWAS) of T2D have now discovered approximately 50 loci related to T2D. The genes contained in these loci will be potential targets of future anti-diabetic drugs, whatever the genes or their products. And these loci have yielded a lot of information that implicates otherwise unknown mechanisms of T2D, which will lead to a deeper understanding of this complex disease. Furthermore, since GWAS of other metabolic traits have also produced many candidate loci, it has become feasible to investigate the function of gene interactions in multiple traits. **Chapter 2** introduces the progress made by GWAS in T2D. This chapter reports one remarkable finding: that most genes associated with T2D are likely associated with beta-cell function instead of IR. If this really proves to be true, then the genetics of IR have remained rather less well-defined. Genes associated with other metabolic traits, such as dyslipidemia, are potential candidates to relate to IR, and could provide important targets for T2D therapy.

Since insulin was discovered, the pharmacotherapy of T2D has made much progress. However, we still cannot prevent the chronic complications that result from diabetes, although we now have many types of drugs to use in treating T2D. New drugs with different mechanisms are being designed and it is hoped they will be more effective than the current drugs. Recent progress in genetics is enabling the search for new genes in diabetes, which should yield new potential drug targets.

2. Demands of prevention of T2D and the future breach

Since we cannot satisfactorily stop the diabetic processes that initiate chronic complications and given the rising prevalence of T2D, the prevention of this disease in high-risk groups has become a crucial issue. Pre-diabetes, which is now defined as including impaired glucose tolerance (IGT, plasma glucose after glucose load in 75g-OGTT is less than 11.1 mmol/L but not less than 7.8 mmol/L) or impaired fasting glucose (IFG, fasting plasma glucose is > 5.6 mmol/L but < 7.0 mmol/L), is believed to confer a high risk of developing T2D[9]. So several population-based studies in individuals of pre-diabetes were performed[10-15].

2.1 Brief history of diabetes prevention studies in pre-diabetes populations

Since pre-diabetes was defined in 2006, most of the intervention studies have

been performed in IGT populations. Lifestyle intervention was observed to prevent the development of T2D in IGT individuals in 1980 in Malmöhus, Sweden[10]. The later population-based study in Sweden, the Malmö study[11], drew the conclusion that the incidence of T2D was much lower in the lifestyle intervention group than in the reference group at the end of the 5-year study period, although it was not a true randomized study. After that, four landmark diabetes prevention studies were performed in China (Da Qing IGT and Diabetes study)[12], Finland (Diabetes Prevention Study, DPS)[13], United States (Diabetes Prevention Program, DPP)[14] and India (India Diabetes Prevention Program, IDPP)[15]. The risk of developing T2D was reduced by 42-58% in the lifestyle intervention groups after 3-6 years and was seen consistently in all these studies. The US DPP also concluded that metformin was effective in preventing T2D. Other pharmacologic interventions, such as acarbose[16], orlistat[17], rosiglitazone and Ramipril[18] were tried to test their effectiveness in preventing T2D, and most of the conclusions so far have been optimistic.

2.2 Can we intervene earlier?

However, the situation to prevent T2D is still difficult from another viewpoint. Although all the outcomes of studies like Da Qing, DPP and DPS proved that lifestyle intervention is still effective after the end of the research period, the percentage of T2D patients were too high. For instance, in the 20-year follow-up study of the Da Qing IGT population in China, the prevalence of T2D reached 93% in the control group and 80% in the lifestyle intervention group[19]. Such results demand that we consider the necessity and possibility of intervening earlier.

One possible high-risk group that should be identified early are individuals with obesity or morbid obesity. The Sweden Obese Subject (SOS) study recently gave the results of their 15-year follow-up[20], which revealed that bariatric surgery can reduce the risk of T2D by 78% in obese individuals. Although bariatric surgery is only appropriate for obese patients whose BMI is >35, it is still exciting to see that this can be another effective measure to prevent T2D.

2.3 Will dyslipidemia be a future target of intervention to prevent T2D?

The fact that most T2D patients do not experience a period of severe obesity means that bariatric surgery cannot be the mainstream measure of T2D prevention, and we should consider other measures and other high-risk candidate groups for

T2D. Since dyslipidemia is closely associated with T2D, IR and other glucose-related traits, is it possible to prevent T2D by intervening in dyslipidemia? The key to this problem is whether dyslipidemia is a causal factor of IR or T2D; this is discussed in detail in **Chapter 3** of this book.

3. Side-effects of insulin and management

The discovery of insulin was a milestone in the history of diabetes therapy, but it also provokes side-effects. At the beginning of its clinical application, the two main side-effects of insulin were hypoglycemia and insulin allergy. How these side-effects can best be treated is still a problem, although the situation has become far better.

3.1 Hypoglycemia and a brief history of insulin preparation

Hypoglycemia occurs when the dosage of insulin is more than enough. In 1922, hypoglycemia occurred frequently in diabetes patients using insulin, because the activity of the insulin was different in each batch. Now insulin production is standardized but hypoglycemia still exists, although it occurs much less frequently and less severely.

The reason that hypoglycemia still exists is that the current model of insulin therapy is not ideal. All we can do in insulin therapy is to mimic the physiological model of how insulin works in normal individuals, while the physiology is a complex system with an automatic negative feedback. When the blood glucose increases because of food or other causes, insulin will be secreted into the circulation to lower it. When the blood glucose concentration decreases to some extent, insulin will stop being secreted. This negative feedback system should include a blood glucose sensor, a control center, and an effector to secrete insulin. But insulin therapy only mimics the function of the effector. Hence a number of preparations and devices have been invented to make the model of insulin therapy similar to the most common model of insulin action.

Generally, we can distinguish two parts in the curve of serum insulin concentration: basal insulin and food-induced insulin. The former is the amount of insulin which is needed for all the physiological actions, and has a low but continuous concentration; the latter is the insulin which is needed to treat the rapidly increased blood glucose concentration because of calorie intake during

a meal, often presented as a peak. To mimic this model of insulin secretion, an insulin pump with a speed-modulating device was invented, which is generally called continuous subcutaneous insulin infusion (CSII) from the insight of injection method. Although it is the best regimen to mimic insulin secretion, its expensive price has limited its application. Another solution to mimic insulin secretion is the basal-bolus regimen, comprising one daily injection of long-acting insulin preparation and three injections of short-acting insulin preparation, one before each meal, which demands appropriate insulin preparations.

However, the insulin introduced in 1922 could only be used as short-acting insulin. Furthermore, this short-acting preparation could not perfectly mimic meal-induced insulin because it was injected subcutaneously, while the insulin secreted by islets was released directly into the portal vein to initiate its function immediately. This is the reason why such insulin preparations need to be injected 20-30 minutes before a meal. Practical basal insulin preparations and better bolus insulin preparations were strongly needed. In the following 90 years, more and more types of insulin preparations were invented to mimic basal or bolus insulin (Box 2). Although none of the current regimens can attain the level of a negative feedback system, hypoglycemia occurs less than before for the same extent of blood glucose control.

Box 2 Brief history of development of insulin preparations

1922	Insulin was first used in clinical medicine
1936	Zinc or protamine was found to be able to prolong the action time of insulin
1946	The commonest intermediate-acting insulin preparation, NPH, was invented
1973	Highly purified insulin preparation was invented
1982	Recombinant human insulin preparation was invented
1994	Rapid-acting insulin analogue Lispro was invented
1995	Rapid-acting insulin analogue Aspart was invented
2000	Long-acting insulin analogue Glargine was invented
2000	Long-acting insulin analogue Detemir was invented
2004	Rapid-acting insulin analogue Glulisine was invented
2011	Long-acting insulin analogue Degludec was invented

3.2 Brief history of insulin allergy

Besides hypoglycemia, another main side effect of insulin when it was introduced in 1922 was allergy[21]. Nearly every patient accepting insulin therapy suffered from local skin symptoms due to insulin allergy. The main causes of insulin allergy are the impurity and animal origin of insulin preparations. Most of those patients suffered from IgE-mediated allergy, also called type I hypersensitivity, and desensitization therapy was invented to alleviate this problem, but the effect was only partly valid. The principle of desensitization was to induce immune-tolerance, but it was not always successful. However, this problem was solved satisfactorily with the development of later pharmaceutical drugs. The appearance of highly purified insulin preparations solved the problem of impurity, while the recombinant technique solved the problem of the animal origin. In the early 1980s it was believed that insulin allergy in humans had been conquered.

However, insulin allergy still occurs after the use of recombinant insulin preparations in spite of having a low prevalence nowadays; unfortunately it seems more difficult to treat. Human-insulin-specific IgE was identified in the sera of these patients and all we can offer is the unsatisfactory desensitization therapy.

Fortunately, two technical advances improved the embarrassing situation of insulin allergy treatment. One was the appearance of various types of insulin analogues, and the other was the popularization of CSII[22]. Rapid-acting insulin analogues seem to decrease the antigenicity through avoiding formulation of hexomers, while long-acting insulin analogues seem to hide the epitopes of insulin preparations. Although CSII was used to treat insulin allergy as early as 1987, it was seldom used for this rare disorder until the 21st century, because CSII is now much more convenient and cheaper. It can provide enough insulin for a diabetic patient at a very slow infusion rate, which avoids attaining the dosage to induce allergy. Clinical application of insulin analogues and CSII mean we can now treat patients with insulin allergy much more effectively.

Generally speaking, insulin analogues and CSII are helpful for diabetic patients with insulin allergy. However, CSII should be used lifelong by these patients, which is often impossible to achieve in many developing countries. Is it possible to use CSII only for desensitization instead of as a long-term therapy regimen? **Chapter 4** describes the effort to manage this regimen.

3.3 Insulin antibody and dysglycemia

In the early period of insulin therapy, hypoglycemia and allergy were very common. And another strange situation also occurred spasmodically: larger and larger insulin dosages were needed to acquire glycemic control even though the patient was not very obese. This was later proved to be due to another type of allergy, type III hypersensitivity induced by insulin. The pathophysiological mechanism is that insulin antibody (IgG) induced by insulin preparation binds insulin to formulate an antigen-antibody complex, so that the circulating isolated insulin is not sufficient to maintain normal glucose concentrations. Similar to typical type I allergy, this disorder of the antigen-antibody complex has become much rarer since recombinant insulin preparations were introduced. However, there are occasionally reports about similar situations induced by IgG, which is needed to treat patients with glucocorticoid, specific immunosuppressants, or plasmapheresis. This rare disease was classified as one subtype of severe insulin resistance syndrome.

Another extreme type of insulin antibody-induced disorder is hypoglycemia. In 1970, Hirata et al reported the first patients with insulin-autoimmune syndrome (IAS)[23]. After that, several hundreds of these patients were reported. The pathophysiological mechanism is also due to the circulating antigen-antibody complex. Insulin antibody – induced by some drugs with sulfhydryl compounds – binds endogenous insulin to form antigen-antibody complex, but this kind of complex can spontaneously dissolve into isolated insulin and antibody, which can induce severe hypoglycemic attacks. Although it has been defined that a patient, without using insulin, could be considered as diagnosed with this disease, some patients treated with insulin rather than with drugs with sulfhydryl compounds have also been classified as having insulin autoimmune syndrome. Most IAS patients had a spontaneous remission after the sulfhydryl compounds were discontinued, but for those patients whose attack was induced by insulin, glucocorticoid therapy was always necessary.

Insulin-induced antigen-antibody complex can cause disorders of dysglycemia. In **Chapter 5**, we describe one case of hypoglycemia with pseudo-negative insulin antibody and one case of hyperglycemia with a zero serum insulin concentration, and we propose a novel classification of dysglycemia induced by insulin-related

antigen-antibody.

4. T2D: the more we know, the more we realize how much we don't know

In the past 100 years, seven Nobel prizes have been awarded in total for contributions to diabetes1 since Banting and Macleod won their Nobel prizes in 1923 for the discovery of insulin, but theirs was the only one that was an achievement in therapy. Although we have found several new effective drugs, none of them can attain the achievement of insulin at that time. This means that we do not have sufficient knowledge to make influential progress now, and we should do more research on the mechanisms of diabetes.

In the past five years, GWAS-related research has provided much more information. But the most different characteristic of this research is that we have not understood most of the information we acquired. It is really a big challenge for scientists to "decode" this information into understandable knowledge. Hence this situation confronts us with more surprises and confusion than before, which not only always makes us feel ignorant but provides us with more opportunities to better understand diabetes. It therefore seems that the next Nobel prize for diabetes will still be awarded for work on the disease mechanisms rather than new treatments.

Another trend in the post-genomic era is the development of other "omics" research, including transcriptomics, proteomics, metabolomics, lipidomics, etc. These "omics" research techniques can be applied to nearly every type of body fluid (plasma, urine, saliva, etc.) and/or tissue (adipose, liver, muscle, etc). Furthermore, as genomics develops, epigenomics appears to give us more information about environmental influences on genes. The emergence of more and more data to be "decoded" seems to have become normal. Preliminary studies of these new subjects have tried to search for biomarkers for the early diagnosis, chronic complications, and management of diabetes.

In the current situation, it is more and more important to focus on systems biology[24]. Although "systems" science dates back to Cybernetics by Norbert Wiener in the 1940s and General Systems theory by Ludwig von Bertalanffy in the 1950s, it was neglected for many years until the information produced by GWAS-related

research. How we can best use all the different kinds of "omics" data to understand the etiology of a disease is one of today's urgent demands, and the goal of systems biology.

Lipid metabolism is also important for human beings, and 14 Nobel prizes have been awarded for related research. Lipid metabolism is closely associated with glucose metabolism, and dyslipidemia is a possible causal factor of insulin resistance, as we describe in **Chapter 3**. Hence, it is naturally being speculated that the combined effects of lipid genes may influence glucose-related traits. Since GWAS-related research provides us with so much data about lipid genes, we can use polygenetic models from insights offered by systems biology to investigate their possible relationship.

In **Chapter 6**, we describe our investigation into the relationship between lipid genes and glucose-related traits. An insufficient number of genes related to IR and the close association between lipids and glucose-related traits seem to imply there is an internal relationship hidden behind some complex traits. We used the information GWAS have provided about lipids, but surprisingly found the opposite pleiotropic effects of lipid genes on glucose-related traits. We set out to determine the relationship between lipid genes and glucose-related traits – and we succeeded – but the unexpected answer shows that many more questions are waiting for us.

5. Summary

Since insulin was first used in clinical medicine, the clinical management of diabetes has been revolutionized. Accompanied by the longer lifespan of diabetic patients, we recognized that diabetes is a much more complex disease than had been expected. But what reveals our ignorance can also inspire us to make more progress, not only in clinical therapy, such as improving the desensitization of insulin allergy, but also in the disease mechanisms, such as the causal relationship between dyslipidemia and IR. Our understanding of diabetes is much deeper than before, especially since the start of the era of genetic studies. The pleiotropic effect of lipid genes on glucose-related traits that is presented in this book is not just a simple reply to the question we asked, but also a new and important topic composed of several questions.

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CHAPTER 2

Genetic insights through genome wide association studies in type 2 diabetes mellitus will lead to new therapeutics

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Abstract: Type 2 diabetes is a disorder of dysregulated glucose homeostasis. Normal glucose homeostasis is a complex process involving several interacting mechanisms, such as insulin secretion, insulin sensitivity, glucose production, and glucose uptake. The dysregulation of one or more of these mechanisms due to environmental and/or genetic factors, can lead to a defective glucose homeostasis. Hyperglycemia is managed by augmenting insulin secretion and/or interaction with hepatic glucose production, as well as by decreasing dietary caloric intake and raising glucose metabolism through exercise. Although these interventions can delay disease progression and correct blood glucose levels, they are not able to cure the disease or stop its progression entirely. Better management of type 2 diabetes is sorely needed. Advances in genotyping techniques and the availability of large patient cohorts have made it possible to identify common genetic variants associated with type 2 diabetes through genome-wide association studies (GWAS). So far, genetic variants on 50 loci have been identified. Most of these loci contain or lie close to genes that were not previously linked to diabetes and they may thus harbor targets for new drugs. It is also hoped that further genetic studies will pave the way for predictive genetic screening. The newly discovered candidate genes for type 2 diabetes can be classified based on their presumed molecular function, and we discuss the relation between these gene classes and current treatments. We go on to consider whether the new genes provide opportunities for developing alternative drug therapies.

Key words: type 2 diabetes, drug targets, genetics, personalized medicine.

INTRODUCTION

The last few decades have witnessed a dramatic increase in the prevalence of type 2 diabetes mellitus, due to changes in food intake combined with less physical exercise, a lifestyle often referred to as Western. The long-term consequences of type 2 diabetes are severe and include cardiovascular disease, retinopathy, neuropathy, nephropathy, and diabetic foot disease. While it has been estimated that worldwide around one billion people are obese, over 180 million people suffer from type 2 diabetes and this number is expected to double over the next 25 years. The number of annual deaths due to type 2 diabetes is difficult to estimate because they are often hidden under cardiovascular disease, but the WHO has estimated it to be between 1 and 3 million in 2006. However, since mortality from this disease often occurs many years after its onset, even 3 million deaths is probably an underestimate of the death toll in the near future. Although external factors such as food-intake-related obesity have attracted much attention, the genetic predisposition for diabetes is also important. While the life-time risk for type 2 diabetes in the Western world is around 10%, first-degree relatives of patients have a 20–40% risk for the disease, and concordance rates for identical twins have been estimated to be 57% or higher (up to 90%) for type 2 diabetes in male twins1. The endeavor to find the underlying genes was unsuccessful for many years although hundreds of genetic associations have been described, based largely on candidate genes. Unfortunately, replication was only possible for a very few variants. However, the recent advent of genome-wide association studies (GWAS) holds promise, since such studies have now uncovered a number of common genetic variants related to diabetes for which replication is also possible. These genes had not previously been linked to diabetes and these loci are therefore expected to lead to new insights into the disease mechanisms.

GLUCOSE HOMEOSTASIS AND DIABETES

Diabetes is a state of persistent hyperglycemia, leading to irreversible damage in a number of tissues, especially the retina, the kidney glomeruli, neural tissue and blood vessels. Normally plasma glucose levels are kept within a narrow range, a process referred to as glucose homeostasis. This homeostasis is regulated in a complex way and involves several axes.

After eating a meal, a person's plasma glucose rises temporarily, after which glucose is rapidly taken up by liver and muscle and to a lesser degree by fat tissue, due to the effects of insulin released from the pancreatic β-cells. Under these circumstances, glucose is converted into glycogen, which is then stored in the liver. When plasma glucose levels have dropped, insulin secretion gradually diminishes. In the post-prandial state, a minimum level of plasma glucose is assured by glucose production in the liver, partly due to release from glycogen and partly due to new formation from precursors such as lactate and the amino acids alanine and glutamine. Insulin has a number of other effects, including lipid storage in adipocytes, and it even increases DNA replication and protein synthesis. The regulation of insulin release is complex; apart from the prevailing plasma glucose level, release from various peptides from the intestine (e.g., glucagon-like peptide 1, GLP1) after a meal and feedback mechanisms ensure adequate insulin release.

Type 2 diabetes is characterized by the combination of disturbances in insulin secretion, and an impairment of the effects of insulin, so-called insulin resistance, which is often related to obesity. Insulin resistance is caused by defects in the signaling pathways that process the insulin signal in its target tissues. It has often been stated that the disease starts with insulin resistance accompanied by raised insulin and glucose levels. At a later stage, we presume β-cells undergo further damage and apoptosis, and are not able to keep up with the demand for insulin release, which then results in higher glucose levels. At least one of the pathways involved in insulin secretion, β-cell regeneration, β-cell survival, or β-cell development are important in determining the vulnerability of the β-cell pool in insulin-resistant conditions. Studies have shown decreases in β-cell function prior to the augmentation of plasma glucose in normal glucose-tolerant first-degree relatives of type 2 diabetes subjects; in these studies these relatives had normal insulin sensitivity[2,3]. While obesity is a major risk factor for diabetes – around 50% of obese subjects will develop type 2 diabetes at some stage (depending on the age when they became obesity), it should be noted that 20% of type 2 diabetes subjects are not obese. It thus seems that obesity is a major risk factor for developing type 2 diabetes, but that it is the vulnerability of the β-cell pool which determines whether obesity in fact triggers type 2 diabetes.

Table 1. Genes Associated with Diabetes: Overview of Their loci, Risk SNPs, Candidate genes, Expressed Tissue, Functions, and Related Medication

Chr	Locus and Risk SNPs	Candidate Gene for type 2 diabetes	Expressed tissue (up tissue database)	Proposed function(s) for gene product	Drug(s) affecting the same pathway as the diabetes gene
1	1p12 rs10923931-T	NOTCH2	Brain, Epithelium, Mammary tumor, Placenta, T-cell, Testis	Growth and development; transcription factor; membrane receptor	Unknown
		ADAM30	Testis	Membrane-anchored protein	
2	2p16.1 rs243021-A	BCL11A	Brain, Embryo, Fetal brain, Lymph, Tonsil	Unknown	Unknown
2	2p21 rs7578597-T	THADA	Amygdala, Brain, Pancreas, Skin, Testis, Uterus	Apoptosis	Unknown
2	2p25 rs35927125-G	KLF11†	Brain, Erythroid cell, Hepatoma, Pancreas,Placenta	regulation of Pdx-1 transcription to influence beta cells activity	Unknown
2	2q23.3 rs7560163-C	RBM43	Tongue	Unknown	Unknown
		RND3	Fetal brain, Kidney, Placenta, Spleen	Binding GTP but has no GTPase activity	
2	2q24.2 rs7593730-?	RBMS1	Muscle, Placenta	Unknown	Unknown
		ITGB6	Keratinocyte, Pancreas, Skeletal muscle	Unknown	
2	2q24.3 rs3923113-A	GRB14	Skin	Interacting with insulin receptors to produce an inhibitory effect on insulin receptor signaling	Unknown
2	2q32	NEUROD1‡	Eye, Retina, Rhabdomyosarcoma	Growth and development; transcription factor that activates several genes including insulin and is important for early β-cell development	Unknown
2	2q36.3 rs7578326-A	IRS1	Epithelium, Eye, Skeletal muscle	Being phosphorylated by insulin receptor tyrosine kinase, an important component in insulin signaling pathway	Unknown
3	3p14.1 rs831571-c	PSMD6	Bone marrow, Lymph, Mammary cancer, Ovary, Placenta, Testis	Unknown	Unknown

to be continued

continued

Chr	Locus and Risk SNPs	Candidate Gene for type 2 diabetes	Expressed tissue (up tissue database)	Proposed function(s) for gene product	Drug(s) affecting the same pathway as the diabetes gene
3	3p14.1 rs4607103-C	ADAMTS9	Brain, Fetus	Cleavage of proteoglycans	Unknown
3	3q27.2 rs1470579-C	IGF2BP2	Colon adenocarcinoma, Pancreas, Pancreatic islet	Growth and development	Unknown
3	3q27.3 rs16861329-G	ST6GAL1	Liver, Lymph, Placenta, Skin, Spleen, Thymus	Catalyze the transfer of sialic acid from CMP-sialic acid to galactose-containing substrates normally found in the Golgi	Unknown
3	3p25 rs1801282	PPARG	Adipose, Adipose tissue, Bone marrow, Colon carcinoma, Heart, Placenta	Nuclear receptor (transcription factor) that regulates adipocyte differentiation	Thiazolidinediones
4	4p16.1 rs1801214-T	WFS1†	Amygdala, Brain	Apoptosis; Endoplasmic Reticulum stress pathway activation	Unknown
4	4p16.3 rs6815464-C	MAEA	Embryo, Lung, Spleen, Thymus	Mediating the attachment of erythroblasts to macrophages	Unknown
5	5q13.3 rs4457053-G	ZBED3	Skin, Spleen	Unknown	Unknown
6	6p21.2 rs9470794-C	ZFAND3	Pancreas, PCR rescued clones	Unknown	Unknown
6	6p21.2 rs1535500-T	KCNK16	Kidney	Potassium channel proteins	Sulfonylurea derivatives
6	6p22.3 rs10440833-A	CDKAL1	Placenta,Testis,Trachea	Growth and development/Proinsulin to insulin conversion	Unknown
6	6q13 rs1048886-G	C6orf57	Whole body	Unknown	Unknown
7	7p15.1 rs849134-A	JAZF1	Amygdala, Brain, Testis	Cell cycle regulation; transcriptional repressor	Unknown

to be continued

continued

Chr	Locus and Risk SNPs	Candidate Gene for type 2 diabetes	Expressed tissue (up tissue database)	Proposed function(s) for gene product	Drug(s) affecting the same pathway as the diabetes gene
7	7p15.3-p15.1	GCK‡	Lung, Pancreas, Placenta	Catalyzes reaction from glucose to glucose-6-phosphate	Unknown
7	7q32.1 rs6467136-G	GCC1	Brain, Cervix carcinoma, Hepatoma, Skin, Uterus	Peripheral membrane protein	Unknown
		PAX4†	Colon, Insulinoma, PCR rescued clones, Placenta	Pancreatic islet development	
7	7q32.3 rs972283-G	KLF14	Epithelium	Part of a transcriptional co-repressor complex	Unknown
8	8q22.1 rs896854-T	TP53INP1	Lung, Thymus	interact physically with p53 and regulatep53 transcriptional activity	Unknown
8	8q24.11 rs3802177-G	SLC30A8	Amygdala, Brain, Lung, Pancreas	B-cell ion homeostasis and insulin secretion; cellular efflux of Zn^{2+} ions/Proinsulin to insulin conversion	Sulfonylurea derivatives
9	9p21.3 rs10965250-G	CDKN2A-2B	Hematopoietic, Skin, Thyroid carcinoma/ Heart, Skin	Cell cycle regulation	Unknown
9	9p24.1 rs17584499-T	PTPRD	Brain, PCR rescued clones, Placenta	A receptor-type of the protein tyrosine phosphatase (PTP) family associated with insulin signaling	Unknown
9	9p24.2 rs7041847-A	GLIS3†	Brain,Testis	Transcription factor, regulation of the development of pancreaticβ-cells and insulin gene expression	Unknown
9	9q21.31 rs13292136-C	CHCHD9 (CHCHD2P9)	Whole body	Unknown	Unknown
9	9q34.3	CEL‡	Colon, Mammary gland, Milk, Pancreas	Glycoprotein that is important in regulation of cholesterol metabolism	Unknown
10	10p13 rs12779790-G	CDC123	Bone marrow, Fibrosarcoma, Foreskin, Lymph	Cell cycle regulation	Unknown
		CAMK1D	Pancreas	Regulation of granulocyte function	

to be continued

continued

Chr	Locus and Risk SNPs	Candidate Gene for type 2 diabetes	Expressed tissue (up tissue database)	Proposed function(s) for gene product	Drug(s) affecting the same pathway as the diabetes gene
10	10q22.1 rs1802295-A	VPS26A	Brain, Colon, Umbilical cord blood	A component of the retromer complex, involved in retrograde transport of proteins from endosomes to the trans-Golgi network	Unknown
10	10q23.33 rs5015480-C	HHEX	Bone marrow, Breast, Leukemia, Myeloid leukemia cell, Peripheral blood monocyte, Uterus	Growth and development; transcription factor	Unknown
		IDE	Brain, Testis	Termination of the response to insulin	
10	10q25.2 rs7903146-T	TCF7L2	Fetus, Gastric carcinoma, Uterus	Wnt signaling/Proinsulin to insulin conversion	Unknown
11	11p15.1 rs5215-C	KCNJ11†	Brain, Breast, Ovary, Placenta, Spleen	B-cell ion homeostasis and insulin secretion	Sulfonylurea derivatives
11	11p15.1	ABCC8‡	Brain, Foreskin, Pancreas, Pancreatic islet	B-cell ion homeostasis and insulin secretion; ATP-binding cassette transporter that modulates ATP-sensitive potassium channels and insulin release	Sulfonylurea derivatives
11	11p15.5 rs231362-G	KCNQ1	Colon, Heart, Kidney, Pancreas, Pooled	B-cell ion homeostasis and insulin secretion	Sulfonylurea derivatives
11	11q13.4 rs1552224-A	CENTD2 (ARAP1)	Brain, Epithelium, Pancreas, Spleen	Regulate the cell-specific trafficking of a receptor protein involved in apoptosis	Unknown
11	11q14.3 rs1387153-T	MTNR1B	Ovary, Retina	An integral membrane GPCR for melatonin	Unknown
12	12q14.3 rs1531343-C	HMGA2	Aorta endothelial cell, Hepatoma	A transcriptional regulating factor in adipogenesis and mesenchymal differentiation	Unknown

to be continued

continued

Chr	Locus and Risk SNPs	Candidate Gene for type 2 diabetes	Expressed tissue (up tissue database)	Proposed function(s) for gene product	Drug(s) affecting the same pathway as the diabetes gene
12	12q21.1 rs7961581-C	TSPAN8	Colon carcinoma, Human small intestine, Liver	Glycoprotein involved in the mediation of signal transduction	Unknown
		LGR5	Placenta	Unknown	
12	12q24.31 rs7957197-T	TCF1† (HNF1A)	Liver	Growth and development; A transcription factor required for the expression of several liver-specific genes, important for Wnt signaling	Unknown
13	13q12.1	PDX1‡	Pancreatic islet	Growth and development; nuclear protein that acts as a transcriptional activator of several genes including insulin and is important for early β-cell development	Unknown
13	13q31.1 rs1359790-G	SPRY2	Brain,Muscle,Skin	Inhibits growth factor-mediated, receptor tyrosine kinase-induced, MAPK signaling	Unknown
15	15q22.2 rs7172432-?	C2CD4A-4B	Colon/ Mammary gland	Nuclear factor related with inflammation	Unknown
15	15q24.3 rs7178572-G	HMG20A	Uterus	Unknown	Unknown
15	15q25.1 rs11634397-G	ZFAND6	Hypothalamus, Kidney, Uterus	Unknown	Unknown
15	15q26.1 rs2028299-C	AP3S2	Brain, Colon, Muscle, Skin	A clathrin-associated adaptor complex that may be involved in vesicle transport and sorting	Unknown
15	15q26.1 rs8042680-A	PRC1	Epithelium, Kidney, Placenta	Cytokinesis	Unknown

to be continued

continued

Chr	Locus and Risk SNPs	Candidate Gene for type 2 diabetes	Expressed tissue (up tissue database)	Proposed function(s) for gene product	Drug(s) affecting the same pathway as the diabetes gene
16	16q12.2 rs11642841-A	FTO	Brain, Cervix, Eye, Lung	Associated to obesity	Unknown
17	17p13.3 rs391300-G	SRR	Brain, Fetal brain cortex, Melanoma	Alter glutamate signaling and affect insulin or glucagon secretion	Unknown
17	17cen-q21.3	TCF2† (HNF1B)	Colon, Liver, Thalamus	Growth and development; transcription factor that forms a complex with the product of TCF1 important for Wnt signaling/Cell cycle regulation	Unknown
19	19q13.11 rs3786897-A	PEPD	Epithelium, Hepatocyte, Kidney, Liver, Mammary cancer, Placenta, Skin, Uterus	Enzyme serves an important role in the recycling of proline	Unknown
20	20q13.12 rs6017317-G	FITM2	Unknown	An evolutionarily conserved family of proteins involved in fat storage	Unknown
		R3HDML	Unknown	Unknown	
20	20q13.12 rs4812829-A	HNF4A†	Kidney, Liver	Growth and development; transcription factor	Unknown
X	Xq28 rs5945326-A	DUSP9	Kidney, Liver, Lung, Placenta	Specificity protein phosphatase for members of the ERK family of MAP kinases	Unknown

Genes included in the list are involved in type 2 diabetes, Maturity Onset Diabetes of the Young (MODY), Permanent Neonatal Diabetes Mellitus (PNDM) or selected syndromic forms of diabetes (4-8, 11-39). The cut-off p-value for the inclusion of type 2 diabetes genes identified by GWAS is 5×10^{-8}. †Genes involved in both monogenic diabetes and type 2 diabetes. ‡Genes merely involved in monogenic diabetes. Candidate genes for type 2 diabetes and their loci and risk SNPs are derived from Catalog of Published Genome-Wide Association Studies of the National Human Genome Research Institute (http://www.genome.gov/gwastudies/index.cfm?pageid=26525384#searchForm). The data of expression of genes in up tissues are derived from David Bioinformatics Resources 6.7 of National Institute of Allergy and Infectious Diseases (NIAID), NIH (http://david.abcc.ncifcrf.gov/home.jsp). And the proposed functions for gene products are derived from a few functional research literatures and the relationship with pathways about type 2 diabetes, most of which are still needed to be further investigated.

Although monogenic forms of diabetes have been found (Table 1) (reviewed in[4]), the majority of cases of type 2 diabetes do not show inheritance as a Mendelian trait, but rather as a genetically complex disorder in which genetic variants predispose individuals to develop the disease. Twin studies have revealed concordance rates of over 50 % (and reported up to 90%) for type 2 diabetes in identical twins[1], which still leaves a substantial role for environmental factors, such as excess food and limited physical activity. The rapid rise in diabetes prevalence over the last few decades strongly suggests that genetic variants involved in type 2 diabetes are interacting with environmental factors.

Candidate-based association studies

Several candidate gene association studies have been performed to identify genes involved in type 2 diabetes. These studies test some of the genetic variants in a gene that is a strong candidate for being involved in the disease. Although a few genes have been identified in this way, in general these studies have not been very successful because the results could not be replicated and the p-values for association of the genetic variants were moderate.

Obviously, the few diabetes genes that were identified and replicated in candidate-based association studies had already been linked to a diabetic phenotype, since the tested genes were mainly selected on the basis of their function and their potential relationship with diabetes. The genes identified by candidate gene approaches were found to be involved in rare, monogenic forms of diabetes. The TCF2 gene was linked to maturity-onset diabetes of the young (MODY) 5[5], while WFS1 gene mutations led to Wolfram (or DIDMOAD) syndrome, a rare, lethal, neurodegenerative disorder that includes diabetes insipidus, diabetes mellitus, optic nerve degeneration, and inner ear deafness[6]. The KCNJ11 gene is related to permanent neonatal diabetes mellitus (PNDM), a rare form of diabetes starting before the age of 6 months[7]. Finally, mutations in PPARγ can lead to insulin resistance, hypertension, and lipodystrophy[8]. Although the molecular impact of mutations in these genes is not yet fully understood, it is likely that mutations in them will have differing effects and that mutations involved in type 2 diabetes will have a milder impact on human physiology than those involved in monogenic diabetes.

Genome-wide association studies (GWAS)

Recent advances in genotyping techniques and the collection of large, type 2 diabetes patient cohorts have made it possible to perform hypothesis-free genome-wide association studies (GWAS) to identify common genetic variants that increase susceptibility to type 2 diabetes. The human genome harbors around 3 billion base-pairs, which contain at least 3 million common single nucleotide polymorphisms (SNPs) according to the International HapMap Project. However, alleles represented by SNPs that are close together have often stayed on the same chromosome in further generations, forming a so-called haplotype. This implies that when one variant has been typed, we know the genotype of a set of other variants that surround the initial genetic marker; in genetic terminology it is said that the SNPs that stayed together on a chromosome are in high linkage disequilibrium. It has been estimated that, in a Caucasian population, assessing 500,000 SNPs will detect around 80% of the common genetic variation. GWAS typically involve the assessment of such numbers of SNPs determined in large case-control studies for association with a disease or with a so-called "phenotypic trait", i.e., type 2 diabetes. It should be noted that GWAS identify association of a genetic locus, and not directly of a gene. It is likely that the most associated SNPs in a genomic region are not the causal variant, but that the disease-producing SNP is in high linkage disequilibrium with the "associated" SNPs. It is also possible that a SNP, even when located in a gene, can influence the expression of a nearby gene located several thousand base-pairs or more away[9]. It is therefore difficult to determine which gene is responsible for the association signal in a GWAS with full certainty. A detailed description of a GWAS is reviewed by McCarthy et al[10].

The genome-wide approach has been very successful for type 2 diabetes, leading to the identification of quite a few common genetic variants associated with the disease lying near genes that had not previously been associated with a diabetic phenotype (Table 1)[11-38]. In the near future this number will probably further increase and a large part of the genetic variation that confers susceptibility to type 2 diabetes will be unveiled. At present, the total effect of the identified genetic variants only explains 10 to 15% of the total type 2 diabetes heritability[39-41]. This small explained percentage of the total type 2 diabetes heritability indicates that the

largest proportion of genetic variation involved in type 2 diabetes still await their discovery. Unveiling this so-called missing heritability[42,43] likely will lead to more interesting discoveries about genetics of type 2 diabetes.

Functions of candidate genes for type 2 diabetes

As discussed above, type 2 diabetes is a disease characterized by impaired β-cell secretion of insulin, in combination with resistance to insulin in its target tissues. Both insulin secretion and insulin sensitivity are influenced by genetic and environmental factors[44]. Genes that harbor variants associated with diabetes can thus be expected to exert their effect through one of these pathways.

Most genes that play a role in monogenic diabetes are important for pancreatic β-cell growth and/or function (Table 1). Genetic variants in three (KCNJ11, TCF2, WFS1) out of four replicated genes for type 2 diabetes found by candidate-based genetic association studies are also related to decreased β-cell function. The best characterized gene is KCNJ11 which encodes a member of a β-cell potassium channel involved in insulin secretion[45]. Mutations in TCF2 are associated with insulin secretion[46], while knock-out mutations in WFS1 have been found to lead to Endoplasmic Reticulum (ER) stress and subsequent apoptosis of pancreatic β-cells[47]. The protein product of the fourth gene identified by a candidate gene approach, PPARγ, is involved in adipocyte differentiation and function (reviewed in[48]). Since rare mutations in this gene lead to insulin resistance and lipodystrophy, variations in this gene are likely to contribute to type 2 diabetes susceptibility through altered insulin effects in adipose tissue.

To explore the functions of the newly discovered genes, various studies have investigated their roles in determining sub-phenotypes of type 2 diabetes, such as peripheral insulin sensitivity and β-cell insulin secretion, and the genetic variants identified in GWAS (except KCNQ1 because variants in this gene have only recently been identified)[15,49-88]. In general, these studies indicate that the genetic variants in the genes identified by association studies mainly act through interference with insulin secretion instead of peripheral insulin sensitivity, and are thus similar to the genes identified through candidate gene approaches and in monogenic diabetes. However, insulin secretion and insulin sensitivity are related through complex and poorly understood mechanisms. Expression studies have

revealed that most of the newly discovered candidate genes for type 2 diabetes are expressed in multiple cell types throughout the body and that the expression of some of these genes under diabetic conditions is unaltered in the pancreatic islets whereas it is different in other tissues (reviewed in [89]). Functional studies of the molecular mechanisms by which most genetic variants lead to impaired insulin secretion and the use of animal models have been quite limited but are sorely needed[90].

In the past five years, GWASs have led to the discovery of 50 new candidate genes related to type 2 diabetes. However, it is still possible that there are mutations in genes that affect other axes of the underlying mechanisms of diabetes. It has been proposed that multiple hits in several diabetes pathways generally occur in subjects destined to develop type 2 diabetes. Some genes related to insulin resistance have been found by GWAS, although the number of genes related to β-cell failure is much larger. Thus, genetic variation associated with type 2 diabetes seems to be more prominently involved in β-cell failure than in insulin resistance.

Genetic subgroups

1. Genes related to β-cell failure

It can be proposed that some of the candidate genes for type 2 diabetes related to β-cell failure can be grouped into four subgroups based on what is known about their molecular function, see Fig. (1) (Some candidate genes are not included in this figure because their functional relation to diabetes is totally elusive). First, KCNJ11, KCNQ1, ABCC8, KCNK16 , SLC30A8, SRR, ADAMTS9, MTNR1B, CAMK1D, CENTD2, DUSP9,BCL11A, PRC1 and CHCHD9 gene products are probably involved in cellular ion homeostasis and insulin secretion. A second group of genes – TCF7L2, TCF1, TCF2, HHEX / IDE, IGF2BP2, CDKAL1, GLIS3,and NOTCH2 –is likely to be involved in the growth and development of the pancreas, which likely influences the overall capacity of the pancreas to secrete insulin. Of this group TCF7L2, TCF1, and TCF2 are important in Wnt signaling (reviewed in literature[91]). In line with the presence of this group of type 2 diabetes candidate genes, some genes whose deficiency can induce MODY, such as NEUROD1, PDX1, HNF4A, and PAX4, are also related to pancreas growth and development. A third group is related with cell cycle events: JAZF1 and TCF2 have both been

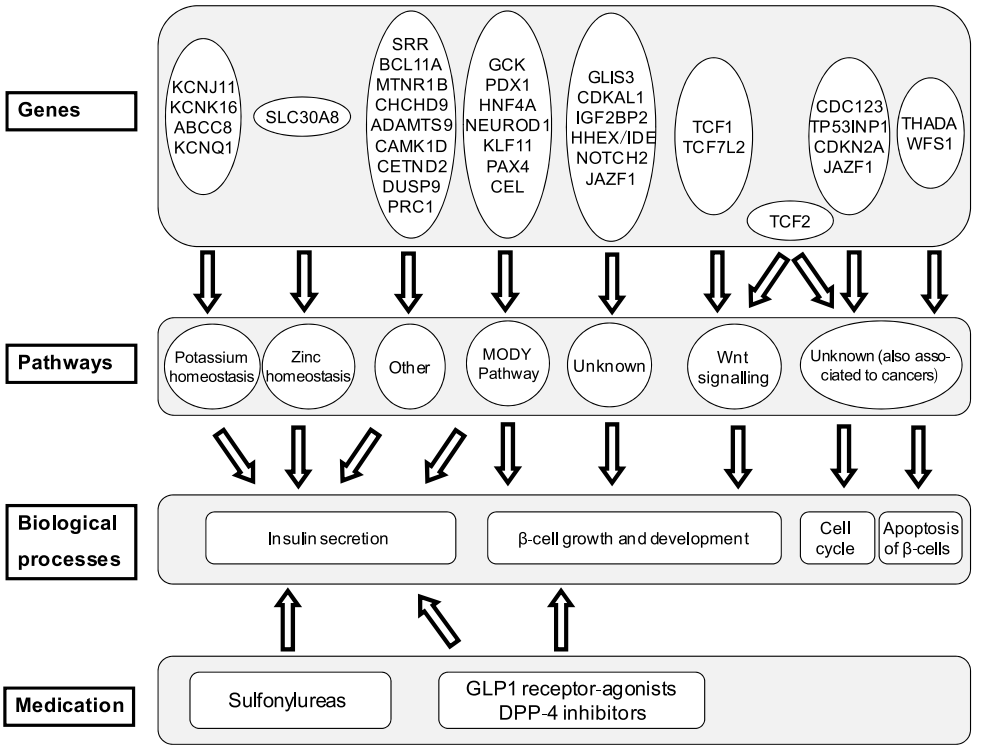

Fig. (1). Classification of candidate genes for type 2 diabetes with a potential role in β-cell function.

associated with prostate cancer and these genes might play a role in the regulation of the cell cycle[92-94]. CDKN2A, TP53INP1, and CDC123 also play a role in the cell cycle. Finally, THADA and WFS1 are thought to be involved in the apoptosis of β-cells. Genes involved in the cell cycle and apoptosis of β-cells might play a role in diabetes by dysregulating the response of β-cells when a higher insulin output is called for when insulin resistance is present.

2. Genes related to insulin resistance

Several genes associated to type 2 diabetes in GWAS seem to be related to insulin resistance, which can be classified into four subgroups. First, FTO, can be classified as a separate group amongst the type 2 diabetes candidate genes since it is the only gene that is associated with both obesity and type 2 diabetes[95,96]. In line with this, Fto deficient mice (Fto-/-) have a significant reductionin adipose tissue and lean body mass. Thus, the association between FTO and type 2 diabetes might be driven by obesity. PPARG may be the only member of the second subgroup,

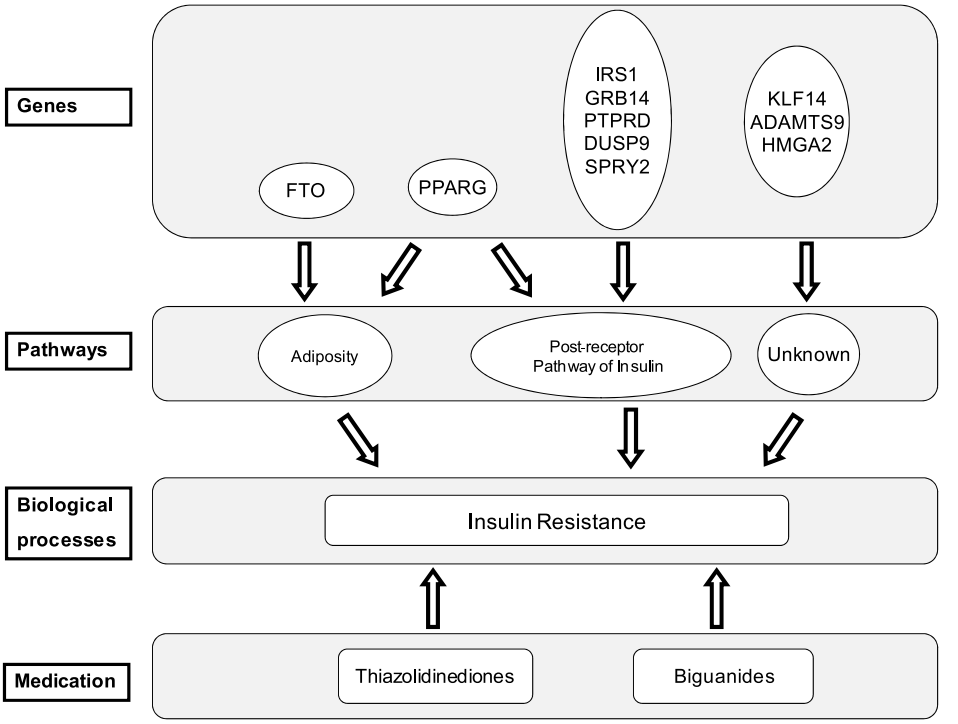

Fig. (2). Classification of candidate genes for type 2 diabetes with a potential role in insulin resistance.

which is believed to influence insulin sensitivity by affecting both adiposity and post-receptor pathway of insulin. A third group includes IRS1, GRB14, PTPRD, DUSP9 and SPRY2, are closely associated with the insulin receptor signaling pathway, and are thought to be related to type 2 diabetes by this mechanism. For example, the protein product encoded by IRS1 is phosphorylated by insulin receptor tyrosine kinase directly. Finally, KLF14, ADAMTS9 and HMGA2 may be also related to insulin resistance [27]; however, knowledge about their mechanism is still limited.

MANAGEMENT OF TYPE 2 DIABETES AND HOW IT IS RELATED TO GENETICS

The corner stone for diabetes management still lies in diet and exercise[97,98]. There is also a slowly expanding list of drugs being used to treat type 2 diabetes, all of which act through one of the pathways important in diabetes pathophysiology.

However, neither changes in lifestyle nor the use of medication are sufficient to cure diabetes, although both interventions can delay the progression of disease. There is therefore an urgent need to develop new medications or strategies to counter the huge increase in cases expected in the future. Since the management of type 2 diabetes with either lifestyle changes, medication or both, is more effective when started at an early stage, improving the techniques for early diagnosis and the opportunities for early intervention will greatly improve the effects of current ways of managing type 2 diabetes.

Pathways important for the function, growth and development of pancreatic β-cells provide obvious drug targets and are already being used, since defects in insulin secretion play a central role in diabetes. Sulfonylurea derivatives are widely used and act through improving β-cell function by closing β-cell potassium channels and thereby enhancing insulin secretion. Some of the genes associated with both monogenic (ABCC8 and KCNJ11) and complex diabetes (KCNJ11, KCNQ1) encode subunits of these potassium channels and accordingly, monogenic diabetes of this type can be well managed with sulfonylureas. SLC30A8 encodes a zinc transporter protein in the β-cell and the function of this gene product might be related to that of the potassium channel genes by regulating ion homeostasis in the β-cell[99].

The actions of various other drugs involve provoking different effects rather than augmenting insulin secretory function: thiazolidinediones enhance insulin activity by acting on adipose tissue, metformin lowers hepatic glucose output, α-glucosidase inhibitors delay digestion and absorption of intestinal carbohydrate, sodium-glucose cotransporter 2 inhibitors increase urinary glucose excretion and pramlintidine delays gastric emptying and inhibits the release of glucagons. GLP1 receptor-agonists or DPP-4 inhibitors have combined actions on food intake by regulating satiety and enhancing insulin secretion in the short term, and β-cell neogenesis and proliferation in the long term (reviewed in [100]). The exact physiological mechanisms that underlie these improvements are, however, unclear. Except for PPARγ and genes involved in pancreas growth and survival, there is no evidence that any of the other genes identified by GWAS are involved in any of these processes. The protein product of PPARγ is likely to be involved in the mechanism targeted by thiazolidinediones because these compounds are

ligands of PPARγ receptors in spite that physiological ligand has not been found[101]. Metformin, the only representative of biguanides, is now believed to be the most widely prescribed oral antidiabetic drug in the world, but its mechanism is too complex to be completely understood. There is some evidence that PPARγ can inhibit hepatic glucose production through the AMPK pathway[102]. However recent research showed that metformin was equally effective to the AMPK deficient mice (AMPK-/-) as to wild type mice, which suggests that metformin could reduce hepatic glucose production through AMPK-independent pathways[103,104]. Although it seems likely that some of the newly discovered candidate genes for type 2 diabetes act in a pathway that is targeted by GLP1 receptor-agonists or DPP-4 inhibitors, more research into both the mechanisms of the medication and the function of the genes is needed.

IMPLICATIONS FOR PREVENTION AND TREATMENT

Genetic screening for prediction and prevention

The effectiveness of current type 2 diabetes management is greatly improved when it is started at an early stage of the disease. If genetic testing could be used to predict type 2 diabetes, preventive measures could be taken and diabetes could potentially be managed more easily. However, the variants associated with type 2 diabetes that have been identified so far only explain a small percentage of the total genetic variation that is thought to be present[39-41]. It is therefore not yet possible to perform accurate predictive genetic testing but, in the near future, research should provide more insight into the opportunities for such testing. Firstly, it is expected that many more common genetic variants will be identified by performing GWAS in other populations and by improving their power and genomic coverage (i.e., more patients included in studies and more SNPs tested). Secondly, performing thorough analyses of the genomic regions that show association to the disease by resequencing large numbers of patients and controls may identify genetic variants that have higher odds ratios than the common genetic variants identified so far. In a GWAS it is unlikely that the functional variant will be identified, but rather one or more common variants to which the disease variant is in linkage disequilibrium, i.e., that the actual causative mutation(s) is in close vicinity to the tested variant. If the

frequency of the causal disease variant is lower than the tested common SNP, we can expect the odds ratio of the causal SNP to be higher and this would make the causal SNP a better candidate to use for genetic testing. One drawback of genetic testing is that many variants have to be tested for each subject, which is laborious and costly at present, but improved genotyping and resequencing technologies will make screening for such variants feasible in the near future.

New opportunities for intervention

Even if the exact functions of the majority of the genes associated with type 2 diabetes are still elusive, they can be broadly grouped into several classes, see Fig. (1) and Fig. (2). When certain genes are involved in the same molecular pathway or physiological process, not only these genes but the entire pathway or process would be an obvious target for new anti-diabetes drugs. In combination with genetic screening, such information could be used to optimize diabetes management by prescribing drugs that act on those pathways that are affected in a patient, and vice versa, it is also possible that altering the activity, be it stimulation or inhibition, of undisturbed processes could improve glucose homeostasis in selected patients. Improved drug treatment in diabetes can be seen in the current management of various MODY subtypes. As described above, mutations in genes involved in β-cell signaling and/or growth in the pancreas have been found to be responsible for MODY subtypes. After these mutations were identified, the management of these diseases was greatly improved by the use of sulfonylurea derivatives, which enhances insulin secretion instead of exogenous insulin[99]. The good response of MODY3 patients to sulfonylurea treatment serves as an excellent example of personalized therapy based on genetic screening.

Evidence that genes identified by GWAS can represent targets for managing type 2 diabetes is provided by the association to this disease of variants in the KCNQ1 and KCNJ11 genes. Both these genes encode proteins that are members of β-cell potassium channels important for insulin secretion and these channels are targeted by sulfonylurea derivates which are already widely used anti-diabetic drugs [99].

Another gene, found through a candidate-based association study and likely to act in the pathway targeted by thiazolidinediones, is PPARγ. On the physiological level no relationships are known between current medications and all the other genetic variants identified in either GWAS or candidate gene association studies.

Therefore all these genes represent new potential targets for intervention.

However, most of the candidate genes for type 2 diabetes have not yet been studied well enough to predict whether interference with their products could be used in managing diabetes. Studies are needed in order to better classify these genes.

Common variants in candidate genes for type 2 diabetes relating to cell cycle events and apoptosis, and representing different alleles than those associated to type 2 diabetes, are also associated with various cancers. In addition, the risks of developing diabetes and prostate cancer are correlated in a complex way: overall diabetes risk and prostate cancer risk are inversely correlated, but while diabetes risk and the risk of developing aggressive prostate cancer show no correlation, these two risks are positively correlated in lean men and in men who undertake vigorous physical activity[105]. Systemic administration of agents interfering with the gene products of diabetes genes that are also associated with cancers could, therefore, be beneficial in treating diabetes, but they might be potentially carcinogenic. Tissue-specific drug targeting or additive medication to overcome the carcinogenic side-effects of such medication needs to be developed to overcome this problem. Hence, cell-cycle related genes are probably not the most promising targets for developing new diabetes treatments, unless cell-type-specific targeting of drugs becomes a reality.

Although the genes containing variants associated with type 2 diabetes that seem to be involved in pancreatic growth and development have not been well studied yet, we can speculate on several possibilities for intervention with such genes. Because most genes in this category are not associated with other diseases, they represent promising intervention targets. Variants in these genes might affect the regenerative or proliferative potential of the β-cell population when there is an increased demand for insulin secretion. In this case it would be possible to use such genes, or the molecular pathways they are involved in, as a target for intervention, in an attempt to correct the poor response of the β-cells and ultimately aiming to improve insulin secretion.

Another possibility is that variants in these genes cause pancreatic damage, which would lead to endocrine malfunction of the pancreas at an early stage of the diabetic development. The possibilities for therapeutic intervention would largely

depend on the severity and reversibility of such a malfunction.

Genetic variants in three genes identified (or confirmed) by GWAS (TCF7L2, CDKAL1, and SLC30A8) are associated with impairment of proinsulin-to-insulin conversion, whereas variants in other genes identified by GWAS are not associated to this[106]. Even if the affected proinsulin-to-insulin conversion is not a primary but a secondary effect of genetic variants in one of the three genes, reconstituting this process would enhance insulin secretion and thereby enhance β-cell function. Although the conversion of proinsulin-to-insulin has long been known to be involved in type 2 diabetes[107], the mechanisms that regulate this process are still undetermined and there is no current therapy that acts by interfering with this process. Association of TCF7L2, CDKAL1, and SLC30A8 to impaired proinsulin-to-insulin conversion has provided evidence that these genes are somehow related to this process and this clears the way for functional research and development of new medication.

CONCLUSIONS

GWAS are an important tool for identifying genetic variation and they have been very successful in finding 50 genetic loci involved in type 2 diabetes. Genotyping techniques will continue to improve and patient cohorts will become larger, which will lead to more genetic variants being identified in the near future. Although the true causal SNPs are often not known, it is anticipated they will be uncovered by deep sequencing of the genomic regions containing association signals. These advances will help elucidate a higher percentage of the total genetic variation, and improve opportunities for genetic screening and personalized diabetes care. Functional studies and animal models harboring specific gene deletions will be needed to study the role of such mutations, while genetic studies using sharply defined endophenotypes will greatly enhance our knowledge about the candidate genes for type 2 diabetes identified by GWAS.

At present, it is too early to expect results from GWAS to lead to new therapies. Nevertheless, it is clear that genetic studies are crucial to dissecting the mechanisms underlying the biological processes and to finding ways to intervene with them, as they provide important clues for directing the focus of functional research. The ongoing avalanche of genetic information about type 2 diabetes will

thus pave the way to further research in the field and has already yielded important information to aid the development of new therapeutic strategies.

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CONFLICT OF INTEREST

The author(s) confirm that this chapter content has no conflict of interest.

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CHAPTER 3

Dyslipidemia as a Causal Factor in the Development of Insulin Resistance

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Abstract

Insulin resistance often occurs with dyslipidemia as part of the metabolic syndrome and the current dominant paradigm is that insulin resistance leads to dyslipidemia. However, dyslipidemia may also cause insulin resistance; this was postulated 30 years ago and has never been refuted. This review summarizes recent evidence from epidemiological, genetic and intervention studies to readdress this old hypothesis.

In large patient cohorts of diverse genetic origin, high plasma levels of triglyceride and low levels of high-density lipoprotein cholesterol (HDL-C) have been shown to predict insulin resistance, while the same is also true for common APOC3 and LPL polymorphisms. In addition, genetic predisposition to a high triglyceride/low HDL-C, as determined by genome-wide association studies, has been related to an elevated risk for type 2 diabetes. It has further been suggested that lipid-modulating drugs can alleviate insulin resistance. Possible mechanisms as to how dyslipidemia may contribute to insulin resistance include lipotoxicity, endoplasmic reticulum stress, and inflammation.

Establishing whether dyslipidemia plays a causal role in the etiology of insulin resistance is important since it could reveal new avenues for combating type 2 diabetes.

1. Introduction

1.1 Need for new pharmaceutical targets to prevent type 2 diabetes (T2D)

The prevalence of T2D is increasing so rapidly that it has become a serious problem worldwide[1,2]. Since we still have no ideal therapy for T2D or its chronic and serious complications, prevention is of the utmost importance.

This point is clearly illustrated by several large-scale interventional trials in Chinese[3,4], American[5,6] and Finnish populations[7,8], which showed that a considerable proportion of patients with impaired glucose-tolerance (IGT) still developed T2D after several years of follow-up, in spite of apparently effective interventions such as lifestyle changes and metformin use. For instance, in the Da Qing Diabetes Prevention Outcome Study, 80% of IGT individuals in the lifestyle intervention group (diet, or exercise, or diet plus exercise) had developed T2D after 20 years of follow-up, although the lifestyle intervention group did much better than the controls (in which 93% of IGT individuals suffered from T2D after 20 years)4. These findings underscore the urgency to prevent T2D at a much earlier stage.

1.2 Insulin-resistant state as target for preventing T2D

T2D is driven by both insulin resistance (IR) and β-cell dysfunction. It is widely accepted that IR exists many years before overt diabetes, while hyperglycemia often appears with gradually impaired β-cell function (Fig. 1). This typical sequence of pathophysiological processes happens during the natural history in most patients with T2D. However, T2D is a heterogeneous disease, hence the stage of normal glucose tolerance but with IR is the potential target period for prevention, before IGT develops in such individuals.

But what can we do for individuals with normal glucose tolerance but an insulin-resistant state? To provide better prevention, we must first better understand the etiology of insulin resistance.

1.3 Dyslipidemia as a possible causal factor of insulin resistance

The concept of insulin resistance was first postulated in the 1930s[9]. Two types of diabetic patients were differentiated by their blood sugar response to insulin therapy, and the description of the insulin-insensitive type represents the earliest research into insulin resistance. In the 1960s, hypertriglyceridemia was found to be related to IR[10,11], but no compelling evidence for a causal relationship could be

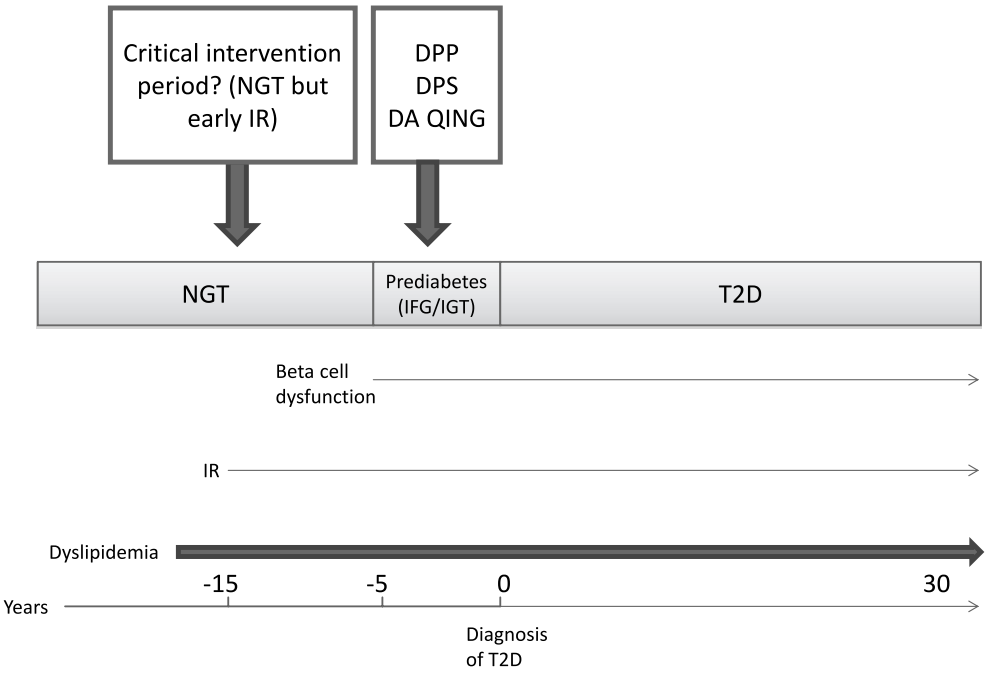

Figure 1. A schematic view of the natural history in most patients with T2D. DPP: Diabetes Prevention Program of United States. DPS: Diabetes Prevention Study of Finland. Da Qing: Diabetes Prevention study of Da Qing, China. All three studies were designed to investigate how to prevent T2D in the IGT population.

discovered. In 1982, Steiner and Vranic postulated the "hypertriglyceridemia-IR-hyperinsulinemia vicious cycle"[12], which emphasized that hypertriglyceridemia could cause IR, while IR and compensatory hyperinsulinemia would aggravate hypertriglyceridemia. They pointed out that it should be possible to prevent T2D by intervening to control hypertriglyceridemia. This was a pioneering hypothesis at that time, although it only concentrated on hypertriglyceridemia due to the then limited studies on blood lipid profiles.

However, at the Banting lecture (American Diabetes Association, New Orleans) in 1988, Reaven coined the term "syndrome X" (later changed to the more popular "metabolic syndrome") to describe the cluster of metabolic disorders including dyslipidemia, hypertension, hyperglycemia, and central obesity, with IR postulated as the common cause[13]. Since then, a lot of evidence has supported the dominant hypothesis that IR can induce dyslipidemia[14]. Compensatory hyperinsulinemia due to IR can induce increased flux of free fatty acids, raise

production of triglycerides in the liver, and decrease high-density lipoprotein cholesterol (HDL-C). Thus, in contrast, by 1990's dyslipidemia was believed to be a bystander rather than a causal factor.

Several years passed and knowledge of dyslipidemia increased dramatically. Recently, new evidence, including results from genome-wide association studies (GWAS), has revealed support for the old "vicious cycle" hypothesis[15,16]. However, we suggest replacing "hypertriglyceridemia" with "dyslipidemia" as it is not yet known which types of dyslipidemia could lead to insulin resistance. We propose a revision of the old hypothesis to: "dyslipidemia is a causal factor of insulin resistance".

Here we should make it clear that we focus on "dyslipidemia" instead of "lipotoxicity"; the latter term, which was coined by Unger nearly 20 years ago[17,18] means ectopic lipid deposition in the cells of non-adipose tissues such as liver, muscle, and heart[19]. In recent years, lipotoxicity has been accepted as one of the main mechanisms behind IR. Although lipotoxicity is associated with hypertriglyceridemia to some extent, they are not one and the same disorder (Box 1). We focus on "dyslipidemia" because we may be able to prevent T2D in the early insulin resistant stage by alleviating dyslipidemia (Fig. 1).

Could all types of dyslipidemia be possible causal factors of IR? On the one hand, it seems that only triglyceride and HDL-C are potential candidates rather than LDL-C. This is based on the Framingham Heart Study showing that hypertriglyceridemia and low HDL-C were more prevalent in T2D patients than in the normal population, while high LDL-C did not differ significantly in these two

Box 1. Differences between dyslipidemia and lipotoxicity

	Dyslipidemia	Lipotoxicity
Definition	Abnormal serum lipoprotein concentrations	Ectopic lipid accumulation
Location	Circulation	In the cell
Quantitative evaluation	Blood test	MRI
Clinical marker	Yes	No
Causing IR	Possible	Yes
Clinical use	Easy	Difficult

groups[20]. This suggested that hypertriglyceridemia and low HDL-C, but not high LDL-C, might be causal factors of T2D. Various types of studies, including some GWAS-related research as shown below, support this inference.

On the other hand, both LDL-C and HDL-C can regulate β-cell function and survival in some in vitro and in vivo studies. LDL-C can decrease glucose-stimulated insulin secretion of β-cells and inhibit proliferation, while infusion of HDL was shown to increase the β-cell insulin secretion and prevent apoptosis[15,21]. Since β-cell dysfunction and IR are tightly connected, it is possible that dyslipidemia might be a causal factor of both β-cell dysfunction and IR. However, because IR usually appears before β-cell dysfunction, it is considered the better target for preventing T2D. Hence, we focus here on the causal relationship between dyslipidemia and IR.

There is strong support for IR stimulating lipogenesis and cholesterol synthesis. Hence, excess amounts of free fatty acids are generated in adipose tissue and transferred to the liver, which results in the overproduction of very low-density lipoprotein (VLDL), increased plasma triglycerides and a reduction of HDL-C levels through the action of CETP (Cholesterol Ester Transfer Protein)[14,22].

However, as this review suggests, dyslipidemia and IR may have a reciprocal, positive feedback relationship, i.e.,they may reinforce each other (Fig. 2). Hypertriglyceridemia and low HDL-C induce IR, while the consequence of IR aggravates dyslipidemia. This “vicious cycle” accelerates the development of IR in the early stages of the development of T2D.

The mechanism through which hypertriglyceridemia and low HDL-C may

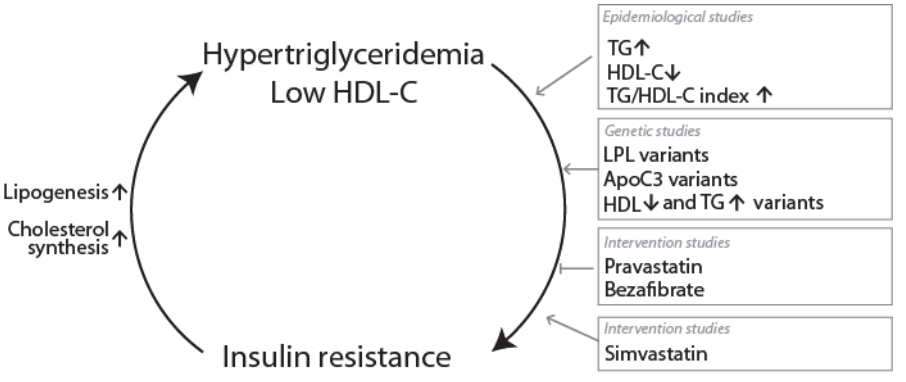

Fig. 2. Possible relationship between dyslipidemia and insulin resistance.

lead to IR still needs to be resolved. Lipotoxicity, inflammation and endoplasmic reticulum (ER) stress are three widely-accepted mechanisms known to induce IR[23-25]. Some human studies have suggested that high serum triglycerides seem to provoke a considerable amount of fatty acids to cells in ectopic lipid storage[26]. Lipotoxicity could thus be the main causal mechanism following dyslipidemia. However, it is also possible that dyslipidemia is a direct cause of inflammation, ER stress, or other mechanisms leading to IR in the absence of lipotoxicity.

In this review, we focus on the evidence from human studies that supports the hypothesis that hypertriglyceridemia and low HDL-C may be causal factors of insulin resistance.

2. Epidemiological studies

If hypertriglyceridemia and low HDL-C are causal factors in the development of IR, one would expect the two types of dyslipidemia to be able to predict future insulin sensitivity. Indeed, the Uppsala Longitudinal Study of Adult Men (ULSAM) has provided evidence that low HDL-C is a long-term predictor of insulin sensitivity[27]. The authors investigated 770 men who had participated in a health survey 20 years earlier and determined their insulin sensitivity through hyperinsulinemic euglycemic clamps. After adjusting for BMI as a confounder, low HDL-C levels proved an independent predictor of IR 20 years later, while triglycerides were not. There have been other longitudinal studies showing that hypertriglyceridemia, low HDL-C levels and/or the serum triglyceride/HDL-C index can predict future risk of developing T2D[28-30]. In the Framingham offspring study[31], 1,004 offspring with baseline pre-diabetes were followed up for seven years and 118 new-onset T2D cases were recorded. Low HDL-C was proved to be one of the key non-glycemic traits that predicted later T2D in pre-diabetes (IGT or impaired fasting glucose, IFG) individuals. Although it is unfortunate that the latter study did not measure insulin sensitivity as an outcome, we can speculate that the deteriorating IR could be a primary cause of the ensuing decline of beta cell function. Combining the results from these epidemiological studies strongly implies that hypertriglyceridemia and/or low HDL-C play a role in the onset of IR, and can be used as one of the long-term predictors of insulin sensitivity.

3. Genetic studies

3.1 Candidate gene studies

Two representative genes encoding proteins in lipoprotein pathways are also known to be involved in IR, thereby supporting a causal link between dyslipidemia and IR. These genes include the Lipoprotein lipase (LPL) gene and Apolipoprotein C3 (ApoC3).

In a case report of a familial lipoprotein lipase (LPL) deficiency, two affected sisters carried loss-of-function mutations in the LPL gene and they both suffered from insulin-resistant diabetes and severe hypertriglyceridemia. After their dyslipidemia was successfully treated by bilio-pancreatic diversion, the patients recovered from diabetes and oral glucose-tolerant tests showed completely normal results[32]. In addition, the glucose infusion rate of hyperinsulinemic-euglycemic clamp studies increased dramatically after surgery, indicating elevated insulin sensitivity.

In another study, 85 heterozygous carriers of either a missense mutation (Gly188Glu) or a splice site mutation (C-->A in position -3 at the acceptor splice site of intron 6) in the LPL gene, were compared with 108 unaffected participants from the same families. The results showed that HOMA-IR (homeostatic model assessment-insulin resistance) index values were significantly higher in carriers than in non-carriers[33]. Furthermore, based on 12 single nucleotide polymorphisms in the 3' end region of the LPL gene, which may change serum triglyceride levels by regulating LPL activity, the risk haplotypes for insulin resistance were discovered and replicated[34]. In hyperinsulinemic-euglycemic clamp studies in 291 individuals from a large, family-based population, the same risk haplotypes were found to be related to the glucose infusion rate[35]. These studies thus provide evidence that loss of LPL gene function affects insulin metabolism. This is likely mediated through its effect on plasma triglyceride levels as well as lipid flux into the tissues.

Another important gene encoding a protein in the pathway controlling VLDL-TG levels is ApoC3, which is related to IR. In a study of 95 Asian Indian men, the T-455C and C-482T polymorphisms of the ApoC3 gene were found to be strongly associated with hypertriglyceridemia[36]. Moreover, it was found that the

prevalence of non-alcoholic fatty liver disease (NAFLD) was 38% among variant-allele carriers, but absent among wild-type homozygotes ($P<0.001$). NAFLD is now known to be a hallmark of IR. The authors concluded that the polymorphisms C-482T and T-455C in ApoC3 are associated with NAFLD and IR. Because ApoC3 is known as a protein inhibiting lipolysis of VLDL, the main effect of ApoC3 mutations is a rise in VLDL-TG, and thus the present study greatly supports our proposed role for VLDL-TG in the etiology of IR.

In conclusion, genetic data on LPL and ApoC3 show convincingly that while the primary cause of the mutations result in dyslipidemia, the secondary consequences lead to IR. In the absence of a direct role for LPL or Apoc3 in insulin resistance, these data do support a causal role of dyslipidemia in the etiology of IR. At the moment, there are no similar examples linking HDL metabolism to insulin signaling.

3.2 Genome-wide association studies

Knowledge on the genetics of complex diseases has developed rapidly in recent years, especially since the completion of the Human Genome Project. The development of highly dense genotyping arrays has led to great success in identifying genetic variants using GWAS. So far, about 100 loci related to dyslipidemia[37,38] have been reported and at least 9 loci for IR have been proposed[39]. Interestingly, several susceptibility loci have been found to be shared by both traits. For instance, both rs972283 for IR and rs4731702 for HDL-C are located near the *KLF14* gene in 7q32.3 and are closely linked. In addition, rs11642841, which is located in the region of the well-known FTO gene for obesity and T2D, is closely linked with rs1421085 for the HDL-C trait. These might suggest common pathways for mechanisms involved in IR and hypertriglyceridemia/low HDL-C, which would be consistent with our hypothesis.

3.3 Mendelian randomization studies

Genetic association studies will not resolve what mechanism links SNP information to dyslipidemia and IR. For this purpose, the Mendelian randomization (MR) approach has been developed. MR is suitable to investigate the causal relationship between clinical observations (biomarker or intermediate/endo-phenotypes) and disease outcomes using genetic information. The basic principle of MR is that if a biomarker (i.e., dyslipidemia in our hypothesis) is a causal factor

for the disease outcome (i.e., insulin resistance in our hypothesis), the genetic variation underlying this biomarker should also be associated with the disease. The association should be consistent with established epidemiological relationships, i.e., SNP alleles that increase total cholesterol (TC), LDL-C or triglycerides (TG) or that decrease HDL-C should be associated with an increased risk of IR. As genotypes are assigned randomly at birth when transmitted from parents to offspring during meiosis, MR is believed to act as a "natural randomized clinical trial". As such, MR studies can help to test whether the association between a risk marker and outcome is causal or whether the marker is raised as part of the disease process (reverse causation)[40]. Following this approach, ten common genetic variants robustly associated with circulating triglyceride levels were studied in 5,637 T2D patients and 6,860 control subjects[41]. There was no evidence that carriers of greater numbers of triglyceride-raising alleles had an increased risk of T2D and higher levels of fasting-insulin or fasting-glucose. The data thus implied that hypertriglyceridemia was not a causal factor of IR41. In contrast, another more recent MR study drew the opposite conclusion that genetic predisposition to low HDL-C or high triglycerides was related to elevated T2D risk[42]. However, both studies had some limitations. The first study covered a limited number of genetic variants (10 loci accounting for only 3-5% of the variation in circulating triglycerides), while the second study covered 25 loci, jointly explaining nearly 10% of the genetic variation, but insulin resistance was not tested. A carefully designed MR study is needed to clarify the causal relationship between dyslipidemia and IR: including all established genetic loci of dyslipidemia, accurate phenotyping of IR cases, and a large sample size.

4. Intervention studies

Over the last few decades many lipid-modulating drugs have been introduced into clinical medicine. Classified according to their main target, these lower LDL-C or lower triglycerides, while recently, HDL-C raising drugs have been developed. In line with our revised hypothesis, we would expect the latter two to alleviate the development of insulin resistance, while the first would not. Unfortunately, since most clinical trials have studied the effectiveness and safety of the drugs in relation to cardiovascular outcomes, data on patients' insulin sensitivity are limited to a few well-documented trials, which we will discuss below.

4.1 LDL-C lowering treatments

So far, statins have been proven to effectively lower coronary artery disease (CAD) risk by reducing serum LDL-C. Their effects on serum triglycerides or HDL-C are moderate. However, over the last ten years, statin use has been reported to lead to an increase in the incidence of T2D. In 2010, a large meta-analysis included 13 statin trials with 91,140 participants[43] and concluded that statin therapy was indeed associated with a slightly increased risk of developing T2D. Another meta-analysis included 16 trials with 1,146 participants and focused on insulin sensitivity, comparing the effects of different statins[44]. They found that pravastatin significantly improved insulin sensitivity, whereas simvastatin made it significantly worse. These results were consistent with earlier data from the West of Scotland Coronary Prevention Study (WOSCOPS)[45], where the authors concluded that the assignment to pravastatin therapy resulted in a 30% reduction of developing diabetes. Without any data on insulin sensitivity, these authors speculated that pravastatin therapy might, at least partly, reduce the development of diabetes by lowering plasma triglyceride levels by as much as 12%.

Pitavastatin is a potent novel statin with LDL-C lowering effects comparable to those of atorvastatin, but with some HDL-C raising effects (5.9-8.2% in general cohort and up to 22.4% in patients with low circulating HDL-C)[46]. In the LIVES study (n = 308), pitavastatin was found to significantly decrease HbA1c in diabetic patients[47]. The authors speculated that pitavastatin might raise HDL-C and thereby improve insulin sensitivity.

Ezetimibe, an intestinal cholesterol absorption inhibitor, can decrease LDL-C by approximately 20%, triglycerides by up to 8%, and raises HDL-C by 1-4%. A small clinical study with 75 participants showed that ezetimibe monotherapy for 12 weeks effectively decreased HOMA-IR, along with improvement of serum LDL-C, triglyceride, and HDL-C levels[48]. Another small study with 45 patients investigated the long-term effect of ezetimibe in patients of NAFLD and found HOMA-IR and other abnormalities of NAFLD also improved significantly[49]. However, larger clinical studies are needed to validate these effects and more in-depth studies should investigate whether insulin sensitivity is improved through controlling dyslipidemia by LDL-C lowering therapies.

Thus, some LDL-C lowering drugs act favorably by reducing HbA1c levels

or delaying the onset of diabetes. However, this beneficial effect appears to be inconsistent among all LDL-C lowering drugs. Moreover, statins have effects on inflammation, which could also lead to improved insulin sensitivity[45]. Other effects may be detrimental, including influence on β-cell function. These differences in response to the different drugs are of great interest to further understand the mechanisms of IR.

4.2 Triglyceride lowering treatments

Fibric acid derivatives (fibrates), which are peroxisome proliferator-activated receptor (PPAR) alpha agonists, reduce serum triglyceride by 25-50% and raise HDL-C by 10-20%, while reducing LDL-C by 5-20%. Bezafibrate was found to be beneficial for delaying the development of pre-diabetes into diabetes in 1990[50]. In subgroups of the Bezafibrate Infarction Prevention (BIP) trial with impaired fasting glucose (n=303), incidence of diabetes was seen to be lower in the bezafibrate treatment group than in controls[51]. In another subgroup of the BIP trial, a group of patients suffering from coronary artery disease treated by bezafibrate (n=1262) had significantly lower HOMA-IR values than the control group (n=1242) after two years of follow-up[52]. Thus, treatment with bezafibrate delayed onset of IR over two years. In addition, it has been shown in a group of 351 patients that treatment with bezafibrate can improve both IR (HOMA-IR) and β-cell function[53]. However, other studies on fibrates (including gemfibrozil, fenofibrate and bezafibrate) reached paradoxical conclusions[54,55]. Some research suggested that the mechanism via which PPAR alpha agonists improve IR was very complex and further basic studies are needed. It is therefore still too early to say whether triglyceride lowering treatment could delay insulin resistance in spite of some supporting evidence.

4.3 HDL modulating treatment

Treatments modulating HDL metabolism are currently under development and only data of small scale studies are available. In a recent, double-blind, crossover study, reconstituted high density lipoprotein (rHDL) infusion in 13 patients was shown to lower plasma glucose levels significantly, while plasma insulin levels increased after 2.5 hours' infusion[56]. Meanwhile, the HOMA-IR measurements in the rHDL and control groups showed no difference. The reduction of plasma glucose could not be explained only by enhanced β-cell function, because the reduction appeared before the elevation of plasma insulin levels. In skeletal muscle

cells it was demonstrated that the AMPK pathway was activated by rHDL[56], which indirectly improved insulin sensitivity of the muscles. Interestingly, CETP inhibitors showed significant beneficial effects on IR in ILLUMINATE trial (n=6661), and were probably related to elevated serum HDL-C levels[57].

In summary, intervention studies have shown that insulin sensitivity is improved by therapies to lower triglycerides and to modulate HDL, but robust insight into the exact mechanisms is still lacking.

5. Using systems genetics to understand disease etiology

Box 2. Looking to the future: what research should be done to test our hypothesis?

1. We need longitudinal studies on large groups of dyslipidemia patients, especially on patients with monogenetic dyslipidemia, and intervention studies on dyslipidemia with insulin sensitivity as the main outcome.
2. We need carefully designed MR studies to clarify the causal relationship between dyslipidemia and IR, preferably including research in diverse ethnic groups.
3. Further elucidation of the underlying etiology of IR will require a comprehensive approach involving genetic variation, intervention of environmental factors, systematic profiling of "omics" data, advanced statistical models, and functional studies in model organisms.

One of the great promises at the current time is that systems genetics approaches will lead to much needed insight into the etiology of IR. In particular, because GWAS studies still leave a large proportion of the heritability unexplained and there is hot debate on the sources of this "missing heritability". These limitations severely hamper the generation of robust mechanistic insights based on causality. Moreover, the current set of GWAS-based genetic loci has only limited power to deliver functional insight. It should be realized that most of the associated SNPs are not in coding regions and they are more likely to be involved in regulatory processes. With the greatly extended regulatory knowledge gained from ENCODE (the Encyclopedia of DNA Elements, http://genome.ucsc.edu/ENCODE/), the lead associated SNPs are, in most cases, in linkage disequilibrium with unknown causal variants[58]. These limitations reduce the power of an MR study and eventually limit our understanding of the disease etiology. Systems genetics approaches seek to understand complex disease traits by integrating systems biology approaches with those of genetics. Thus, in the post-GWAS era, these approaches are becoming a

powerful way of inferring causal roles from genotype to phenotypes. To understand the causal relationship between dyslipidemia and IR, we have to develop novel approaches, such as identifying the causal variants and genes of dyslipidemia from ENCODE and other bioinformatic data, treating those genetic variants as multifactorial perturbations in biological systems, and examining their effect on all aspects and traits related to IR. Subsequently, we should perform functional studies in model organisms and longitudinal studies in biological systems to confirm the findings.

6. Conclusion

Unraveling whether dyslipidemia is a possible cause of insulin resistance is important, as it has many ramifications regarding the early diagnosis and prevention of T2D. LDL-C has been excluded as a relevant factor, since there is no evidence to support a causal relationship with IR. Hypertriglyceridemia and low HDL-C have been shown to be independent, long-term predictors of insulin sensitivity in longitudinal studies, while some genetic studies on genes related to hyperglyceridemia and/or low HDL-C have revealed that these genes are also linked to insulin sensitivity. Furthermore, controlling hypertriglyceridemia and low HDL-C has delayed onset of IR in some intervention studies. Additional genetic, MR and systems biology studies are now feasible and needed to confirm the causal role of hyperlipidemia in IR and its underlying mechanisms. Such studies will lead to urgently needed insight into ways to prevent insulin resistance and the subsequent development of type 2 diabetes.

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CHAPTER 4

CSII as an Effective method of Desensitization Therapy of diabetic Patients with Insulin Allergy

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Abstract:

Objective: To summarize our experience in the application of continuous subcutaneous insulin infusion (CSII) only as a method of rapid desensitization therapy for diabetic patients with insulin allergy and subsequent switching to the regimen of multiple dose injection for a long-time insulin therapy.

Methods: The clinical data of 8 diabetic patients with insulin allergy in PUMC Hospital from April 2008 to December 2011 were retrospectively analyzed.

Results: All 8 patients were diagnosed by case history, skin test, determination of serum specific anti-insulin IgE and reaction to withdrawal of insulin. Five patients accepted traditional injection method of desensitization, while 4 patients accepted CSII with the protocol designed by ourselves (1 patient accepted CSII after failure by the formal method). 4 of the 5 patients accepting the traditional method and all 4 patients accepting CSII succeeded. All patients accepted the regimen of multiple dosage injection as long-term insulin therapy. In a survey of 28 nurses, both experienced nurses and practical nurses preferred to use CSII as the method of desensitization.

Conclusion: It is feasible and effective for diabetic patients with insulin allergy to merely use CSII as a method of rapid desensitization and subsequently switching to regimen of multiple dose injection for a long-term insulin therapy.

Introduction

Patients with poor control of type 2 diabetes mellitus on maximum oral hypoglycemic therapy invariably need insulin therapy. In the adverse reactions of insulin therapy, insulin allergy is still a problem difficult to treat once it happens, although its incidence has dramatically decreased to less than 1% of diabetic patients receiving insulin therapy since the introduction of recombinant human insulin[1]. However, it results in potentially life-threatening immediate or delayed, local and general manifestations[2]. Though some cases suffered from protamine[3], Zinc[4] or meta-cresol[5] of insulin preparations, usually it is persistent IgE-mediated insulin allergy (type 1 allergy)[6], unresponsive to switching to different insulin type or the use of antihistamines, necessitates desensitization[1,6]. Different treatments of unequal efficiency have been proposed, a number of case reports and recent reviews have demonstrated that diabetic patients suffering from insulin allergy can be tolerated with continuous subcutaneous insulin infusion (CSII) since 1987[7-10]. However, nearly all of those cases successfully treated by CSII accepted this type of therapy as the style of long time insulin therapy. Unfortunately, according to insurance policy in China, this expensive treatment is not covered for type 2 diabetic patients whatever they suffered from insulin allergy. Hence we had to attempt to use CSII as a method of desensitization instead of a long-term regimen of insulin therapy so that patients suffering from insulin allergy could spend as little money as possible. We retrospectively analyzed 8 cases of patients with diabetes all presented with local skin reactions after subcutaneous injection of different types of insulin preparations. The aim of this study is to investigate the possibility of adopting CSII as a method of rapid desensitization and then switching to the regimen of multiple dose injection (MDI) for a long-time insulin therapy.

Materials and Methods

We retrospectively analyzed medical records of 8 diabetic patients presented with local skin reactions after subcutaneous injection of different kinds of insulin preparations from clinical wards of endocrinology of the Peking Union Medical College Hospital from April 1st 2008 to December 31st 2011. They were treated with rapid insulin desensitization (4 patients with CSII, 5 patients with traditional rapid insulin desensitization, and among them 1 case experienced both methods). Data

of these patients were shown in table 1. The protocol of therapy had been approved by the appropriate ethical committees related to the Peking Union Medical College Hospital, and we did the insulin desensitization therapy after getting these patients' written informed contents.

Before we started the insulin desensitization therapy, the patients should be evaluated whether insulin was the only therapeutic method to control their blood glucose.

CSII desensitization protocol:

We designed a protocol of CSII application in desensitization therapy according to the principle that immune tolerance might be induced by gradually increasing dosage from extremely small amount, and succeeded in the first pilot case[11]. The protocol was listed as follows in detail. The short action insulin preparation was diluted 10 times (from U100 to U10 by saline). The Minimed insulin pumps were used in all these patients (the first two patients with Minimed 508C, the other three patients with Minimed 712 pump). The basal rate of diluted insulin began with 0.01 IU per hour of insulin, and then increased the basal infusion rate with the speed of 0.01 IU per hour on the first day of desensitization. At the end of the first day, the basal rate would increase to 0.24 IU per hour. If the patient was free of any symptoms of allergy, we would start the basal rate from 0.25 IU per hour on the second day (using the U100 insulin without saline dilution), increased the rate by 0.05 IU per hour, till 1.4 IU per hour at the end of the second day. On the third day of desensitization, we kept the basal rate as 1.0 IU per hour or decreased to 0.5 IU per hour at night to avoid hypoglycemia in older patient, added on the bolus insulin infusion 4-6 IU before each meal with the insulin pump. After that, we gave the bolus insulin by subcutaneous injection using syringe with the basal insulin infusion by CSII at the same time on the fourth day. Finally we stopped using CSII, gave the patient with insulin therapy by multiple subcutaneous injection alternatively.

Results

Diagnosis of insulin allergy:

The standard diagnosis protocol of insulin allergy was exclusion of poor injection technique and reaction to alcohol wipes first. Then insulin allergy was

Table1. The general characteristics of the diabetic patient with allergy to insulin

case	sex	Age (year)	Course of Diabetes (year)	Allergy onset after initiation of insulin	Manifestation of allergy	Insulin selected in therapy	Duration of therapy (day)	Serum total IgE (kU/l)	Specific IgE to porcine insulin (grade)	Specific IgE to bovine insulin (grade)	Specific IgE to human insulin (grade)	Specific IgE to protamine (grade)	Allergy to others
1	F	53	7	10m	Itching, wheal	lispro	37(failed)	63.6NA	23	2NA	23	NANA	NoNo
2	M	68	1	5m	Itching, wheal, induration, pain	Humulin R	3	127	3	3	3	NA	No
3	M	65	12	1w	Wheal, papule, itching	lispro	3	535	3	3	3	0	Penicillin, cefepime, gliquidone
4	M	73	20	8m	Induration, itching	lispro	3	266	0	0	0	0	Levofloxacin, fish, shrimp, green onion, sesame, mushroom
5	F	18	0.5	1m	Pain, itching, rash	lispro	5	510	3	3	3	0	penicillin
6	M	65	10	2w	Redness, induration, itching	Humulin R	5	NA	3	3	3	0	Phenemal, levofloxacin
7	F	54	11	5d	Papule, pain, itching	Novolin R	6	NA	NA	NA	0	NA	Adhesive plaster
8	M	47	4	11d	Induration, itching	aspart	4	136	3	2	2	0	patulin

Note: case 1-4 accepted therapy of CSII for desensitization, while case 5-8 accepted therapy of multiple subcutaneous insulin injection; case 5 was diagnosed as mitochondrial diabetes mellitus. Case 1 was admitted in our hospital twice to accept these two methods of desensitization respectively, and the lower row showed the data of the traditional regimen of multiple dose injection.

Specific IgE to insulin grading: 0: <0.35KUA/L; 1: ≥0.35KUA/L (suspected or mild); 2: ≥0.70KUA/L (moderate); 3: ≥3.5KUA/L; 4: ≥17.5KUA/L; 5: ≥50KUA/L; 6: ≥100KUA/L (grade 3-6: severe).

diagnosed according to the following four respects. First, case history was checked to assure the symptoms and signs consistent with insulin allergy. Second, the description whether the patient's reaction alleviated after insulin was retrieved was carefully reviewed and the clinical manifestations of all 8 cases disappeared after insulin therapy stopped. Third, serum specific anti-insulin Ig E to porcine, bovine, human insulin and specific anti-protamine Ig E in these patients were assessed. Last, Intra-dermal skin tests were performed with several insulin preparations to assist diagnosis of insulin allergy. All of the 8 cases were consistent with those four criteria for diagnosis of insulin allergy and needed insulin therapy inevitably (Table 1). For case 4 and case 7, whose serum specific anti-human-insulin IgE could not be detected, we found they stopped usage of insulin for more than 3 months when they were determined; and serum specific anti-human-insulin IgE of Case 4 attained grade 2 when he was treated by insulin. Porcine regular, human regular and short-acting human insulin analogs (including aspart and lispro) and NPH were used in the intra-dermal skin tests, the volume of all these kinds of insulin preparations was 0.1ml containing 1 unit insulin each, and we used saline as negative control (the volume was also 0.1ml). We did not include protamine, Zn, metacresol et al because these substances for diagnosis or therapy are not available in China. After the intra-dermal skin tests were performed, the situations of erythema and wheal were recorded immediately and carefully.

Selecting one insulin/insulin analogue preparation for desensitization

Intra-dermal skin tests were performed with all types of insulin preparations listed above and the volume of each skin-test solution was 0.1ml containing 1 unit of insulin, and saline was used as negative control. Based on the local skin reactions including the occurrence and disappearance time and the area size of erythema, wheal, induration or pseudopodia, one of the insulin preparations with the weakest reaction was selected as the one for desensitization (usually the regular insulin or short-acting insulin analogue).

Processes of desensitization

According to the rules of desensitization, the process of insulin desensitization was performed under closely monitoring with preparations for emergency interventions in an inpatient setting[11]. There were 4 cases accepted CSII as the method of desensitization therapy while 5 cases accepted traditional desensitization

therapy (multiple dose injection) depending on the willingness of the patients, and one case accepted the former treatment after failing to tolerate insulin by the latter one. We used the pilot CSII desensitization protocol designed by ourselves for all the 4 cases who accepted CSII for desensitization (The protocol was listed in detail above in the Method section). Using this protocol, in the period of 3 days therapy all these 4 patients received CSII desensitization remained free of any symptoms of insulin allergy. We gave the bolus insulin by subcutaneous injection using syringe with the basal insulin infusion by CSII at the same time on the fourth day. After that, they were all successfully switched to insulin therapy by multiple subcutaneous injections with syringe. For other 5 patients, traditional desensitization protocol was applied according to the classical method[1].

Effects of two types of insulin desensitization therapy

All 4 cases who accepted CSII for desensitization were tolerated with the regimen of multiple dose insulin injection while 4 of 5 cases succeeded by traditional desensitization procedure. The unsuccessful one was case 1, and she finally agreed to use CSII protocol after the failure of traditional treatment of desensitization. After 3 days she was free of allergic symptoms and the regimen of insulin therapy was successfully switched from CSII to multiple subcutaneous injections by syringe on the fourth day. After the first following-up for 3-5 months, all these 4 patients using CSII for desensitization were free of any insulin allergic reactions by multiple subcutaneous injection therapy.

The survey of the nurses' opinion of CSII in the insulin desensitization therapy:

The nurses with the experiences of operating insulin pump and those without were asked to fulfill the questionnaire of the convenience and the effectiveness of this method. If they didn't use insulin pump before, they would answer the question, "If you have learned how to operate insulin pump, which kind of desensitization therapy do you prefer?" For the results, 15 nurses with the experience of operating insulin pump were all prefer the desensitization therapy by CSII to multiple injections by syringe. Other 13 practical nurses who finished the questionnaire would like to learn how to operate insulin pump, and then use this method in the desensitization therapy. The reason they preferred CSII therapy in the insulin allergic patients included that: it can mitigate the workload of them, avoid

the stimulation of syringe needle, and decrease the error rate.

Discussion

In the current study, we investigated the possibility of using CSII as a method of rapid desensitization instead of a long-term regimen of insulin therapy for diabetic patients allergic to insulin. The fact that all 4 cases accepting this protocol got the satisfactory effect suggests that CSII as a method of desensitization may be a good choice for those patients.

To use CSII only as a method of rapid desensitization instead of a long-term regimen of insulin therapy might be a good choice in the developing countries. Although CSII was introduced to treat insulin allergy as early as 1987[7], most cases accepted CSII regimen for whole life[9]. Only in 2012, a patient with insulin allergy had been treated by CSII successfully switched to multiple dosage injection one year later, was reported[12]. However, in many developing countries, CSII is still an expensive kind of insulin therapy so that most of the patients cannot pay for its expenses. According to our procedure, it is unnecessary for those patients to use CSII for whole life; it only costs the expenditure for renting an insulin pump for about 3 days. Furthermore, it is also unnecessary for patients to study how to use insulin pump; it could be managed by experienced nurses in the wards. Hence it is more practical to use CSII as only a method of desensitization in developing countries.

CSII is proposed to be an ideal method for treating insulin allergy in many literatures[9,10], but more convincing data are needed to support this opinion. We tried to compare the effect between CSII and traditional regimen of desensitization, but the limited number of those rare cases restricted further analysis by statistics. From our data, it seemed that CSII was at least as effective as traditional regimen of desensitization. It was possible that CSII was superior to traditional regimen theoretically since CSII can make the dosage of insulin increasing more gradually and stably. In many case reports, CSII is always used after traditional regimen does not work. In the current study, there was also one patient who failed by traditional regimen but succeeded by CSII. Furthermore, we can conclude from the simple survey of nurses that CSII as a method of desensitization was widely accepted by nurses.

In the current study, we designed a detailed protocol of CSII desensitization according to the principle of desensitization since most previous literatures did not provide a standard protocol or simply used CSII as common. The practice of this protocol in 4 cases certified its feasibility.

There were some shortcomings in our series of cases analysis. First, it was a retrospectively observational study, but it would be controversial in medical ethics if we decided the regimen randomly instead of based on each patient's will. Second, we could not detect protamine, Zn cation, meso-cresol et al because the relative kits are not available in China. Third, because the sample size was not large enough, we cannot compare the effect of two types of desensitization regimen statistically, although it was the largest clinical study about CSII application in insulin allergy till now. Large sample, well-designed, multi-centered clinical studies are needed to investigate whether CSII will be the first choice of desensitization.

In conclusion, it is possible and effective for diabetic patients to merely use CSII as a method of rapid desensitization and then switch to the regimen of multiple dose injection for a long-time insulin therapy. This procedure is favored by nurses in the clinical ward because of lower workload and error rates. Further studies are needed to evaluate the advantage of CSII in insulin desensitization.

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Declaration of Competing Interests: The authors declare that they have no conflict of interest.

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CHAPTER 5

Insulin as an Antigen of Anti-drug Antibody: Hyperglycemia and Hypoglycemia: a Possible Novel Classification of Insulin Antibody Related Diseases Based on Etiology

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Abstract:

Both anti-insulin antibody induced hypoglycemia and anti-insulin antibody induced hyperglycemia are rare diseases. We provided either a case of them and both were more difficult than typical case. The first case is a patient whose clinical investigation was typical as anti-insulin antibody induced-hypoglycemia but serum anti-insulin antibody was negative; he was diagnosed by column chromatography successfully. The second case is a patient whose daily insulin dosage was about 800IU but serum insulin level was 0mmol/L, finally diagnosed by recovery test. Either case was treated effectively by immunosuppressive therapy. The process of diagnosis suggested that application of laboratory methods would be useful in some rare diseases. A novel classification of insulin-related antibody induced dysglycemia is suggested according to pathogenesis.

Introduction

Autoimmune hypoglycemia is a rare disease. In 1960 Harwood[1] reported on a 34-year-old woman with type 1 diabetes who demonstrated insulin resistance (140 to 180 U insulin/d) but also intermittent episodes of severe hypoglycemia. The patient's serum contained anti-insulin antibody with high insulin binding capacity and a slow rate of dissociation of insulin-antibody complexes. It was postulated that insulin was "stored" at high levels in the serum bound to anti-insulin antibody, and that the slow rate of dissociation of insulin from the complex composed with insulin and anti-insulin antibody resulted in intermittent hypoglycemia. In 1970 Hirata[2] nominated Insulin autoimmune syndrome (IAS, or Hirata's disease) to describe the condition of hypoglycemia, elevated insulin levels, and high levels of anti-insulin antibody in individuals never exposed to exogenous insulin therapy. These patients usually had a history of taking drugs with sulphydryl compounds. Most of the cases were Japanese, and there were over 40 cases[3] in China. In fact, autoimmune hypoglycemia has also been reported[4] in the patients receiving recombinant human insulin therapy. It is the insulin-antibody complex, which releases the free insulin intermittently, directly causes hypoglycemia despite that the antigen is endogenous or extrinsic insulin. Therefore, it is important for diagnosis of autoimmune hypoglycemia that the anti-insulin antibody is positive. Here we report a case that the clinical investigation was similar as autoimmune hypoglycemia but anti-insulin antibody was negative; and we succeeded to confirm the existence of macromolecular protein complex containing insulin in serum by column chromatography.

Meanwhile, the complex composed with insulin and anti-insulin antibody can induce not only hypoglycemia but also severe hyperglycemia, although it is extremely rare. We also reported a case whose daily insulin dosage attained 800IU but serum insulin level was 0mmol/L, finally diagnosed as anti-insulin antibody induced hyperglycemia.

Case 1

A 77-year-old Chinese male patient was admitted to our hospital because of intermittent hypoglycemic attacks for 5 months. The patient had type 2 diabetes mellitus for three years and was on treatment with acarbose until he turned to

insulin therapy because of the partial resection of rectum one year earlier. His fasting blood glucose level (FBG) was 5-7mmol/l and postprandial blood glucose level was 8-11mmol/l by NPH 8IU subcutaneous injection before every breakfast. 6 months earlier the patient felt fatigue, palpitated and perspired with the serum glucose 2.2mmol/l, and the symptoms were relieved after eating some candy. After that the hypoglycemic attacks happened 4-6 times every month, most of which occurred before dawn, and the lowest serum blood glucose level attained 1.8mmol/l. There were still intermittent hypoglycemic episodes after the insulin therapy changed to treatment of nateglinide or arcabose. The patient had taken catopril for 2 years and clindamycin once, which were drugs containing sulfhydryl group. Laboratory test showed HbA1c 6.3%, and anti-insulin antibody negative. When the hypoglycemia attack was confirmed, we found serum insulin and C-peptide levels were very high (see Table 1, 2). Imaging findings including Multi-slice spiral CT perfusion scan did not reveal any tumor in pancreas.

Table 1. Insulin Releasing Test

	Fasting	0.5hr	1hr	2hr	3hr
Blood Glucosel level (mmol/L)	4.42	6.27	11.98	18.31	15.58
Insulin (uIU/ml)	95.90	102.00	107.00	177.00	237.00
C-peptide (ng/ml)	5.26	5.17	6.85	>7	>7

Table 2. Serum insulin, C-peptide and proinsulin levels at the hypoglycemia episode

	Glu (mg/dl)	C-peptide (ng/ml)	Insulin (uIU/ml, CLIA)	Insulin (mU/L, ELISA)	Proinsulin (pmol/L)
1	1.2	3.4	127.84	67.7	33.1
2	2.3	4.0	71.71	43.5	79.3
3	2.3	5.6	167.71	22.4	40.7

Considering the possibility of the false negative of anti-insulin antibody determination, we drew the venous blood of the patient and isolated the serum, and separated the serum by column chromatograph and collect the No.11- 100 dilutions. Then insulin and adiponectin levels of each dilution were determined by

ELISA. The insulin level of No. 17 dilution rose obviously, and the peak value, which attained 65 times than baseline, occurred in the No.22. The peak value of the adiponectin occurred in the No. 16 and No. 27 dilutions (Figure 1). Hence the existence of insulin-bound protein was proved and the complex composed of insulin and anti-insulin antibody was most possible. Therefore, anti-insulin antibody induced hypoglycemia was suspected.

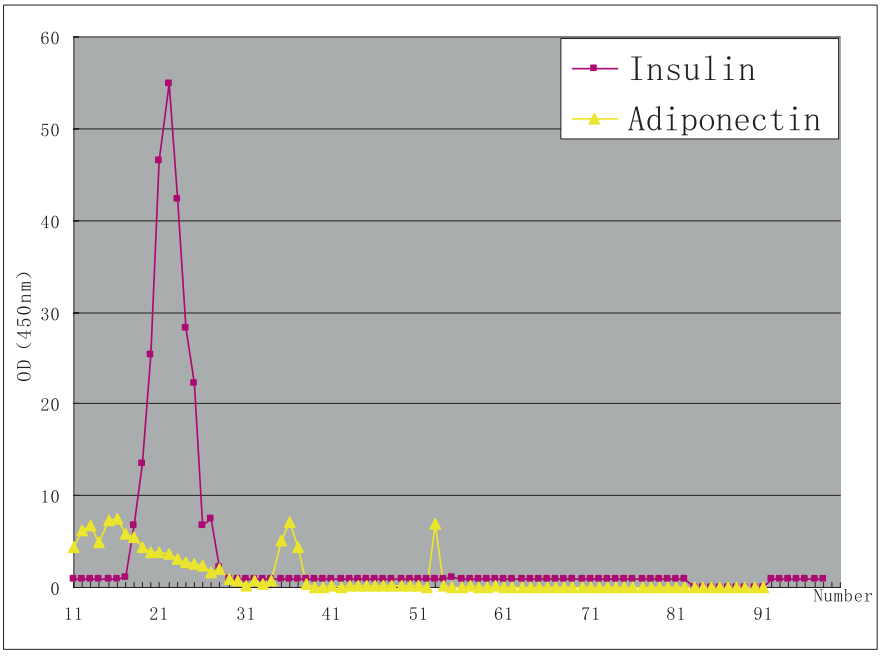

Figure 1. Insulin and adiponectin levels of the serum dilution after column chromatography

After that, the patient accepted prednisone 30mg QD and symptom of hypoglycemia relieved about a week later. We decreased prednisone gradually and stopped it 4 months later. The patient did not have hypoglycemia during 6 months follow-up visit.

Discussion of diagnosis

Autoimmune hypoglycemia is a rare disease. Before autoimmune hypoglycemia is diagnosed, we need to exclude insulinoma and malignant tumor

which also can cause hypoglycemia. In this case, the serum insulin level of the patient was very high when he had hypoglycemia, which indicates insulinoma, but there are not positive findings in pancreatic perfusion CT, ultrasonic endoscope and ^{99m}Tc-octreotide scintigraphy. The sensitivity of perfusion CT to detect insulinoma is nearly 100% these days[5,6]. Considering the high sensitivity of pancreas perfusion CT and the patient is maransis, the insulinoma was nearly excluded. Malignant tumors which lead to hypoglycemia could not explain serum high insulin level. Hence autoimmune hypoglycemia seemed to be the possible diagnosis.

Most cases of autoimmune hypoglycemia are caused by anti-insulin antibody, but we should also be aware of insulin receptor antibody, which can cause hypoglycemia too. Usually insulin receptor antibody is an antagonist to insulin receptor and results in severe insulin resistance; but in very rare situations, it can act as agonist and results in hypoglycemia. The hypoglycemia of the patient is not caused by insulin receptor antibody because he had high serum C peptide. The clinical feature of the hypoglycemia caused by anti-insulin antibody is paroxysmal hypoglycemia, which is caused by unpredictable dissociation of antigen and antibody compound. For this reason, the detection of anti-insulin antibody is very important for making a diagnosis. While we should notice there are many factors affecting the accuracy of anti-insulin antibody determination.

In present, the purpose to detect anti-insulin antibody is for diagnosing type 1 diabetes mellitus instead of autoimmune hypoglycemia. In most hospitals that can run anti-insulin antibody test, ELISA and CLIA have replaced RIA becoming the first choice to determine anti-insulin antibody. In these two methods, insulin special fragment is taken as coating antigen and is coated at tubal wall, the second antibody is anti-IgG-Fc fragment antibody attaching enzyme active group or fluorescence active group; when there is anti-insulin antibody in serum, it will bind with insulin special fragment coated at tubal wall, and then bind with tagged second antibody. After incubation, anti-insulin antibody will anchor at tubal wall and be detected. In type 1 diabetes mellitus, insulin is absolutely deficient, if there is anti-insulin antibody, its amount will be relatively large and test result will be more reliable. But in patient with autoimmune hypoglycemia, the serum insulin level is normal and most anti-insulin antibody bind with insulin, which cause coating antigen (insulin fragment) at tubal wall competes with serum antibody-

insulin compound and inhibits antibody anchoring at tubal wall. Most anti-insulin antibody is discarded in supernatant and the test result is negative. Consequently in autoimmune hypoglycemia caused by anti-insulin antibody, the false negative result using ELISA and CLIA to test anti-insulin antibody is possible.

In this case, we attempt to prove there is large amount of antibody-insulin compound in patient serum, which cause his hypoglycemia. We separate serum proteins according their molecular weight by column chromatography, then test insulin level in each tube and determine the peak value. At the same time, we also test adiponectin level and its peak value. We can see two adiponectin peak values in figure 1, which is in common with the fact that there are two types of molecular weight adiponectin. The insulin peak appears between the two adiponectin peak, which means molecular weight of the under testing substance is between these two kind adiponectin, in another saying, is larger than 150KD, far larger than molecular weight of insulin which is 5.7KD. We deduce this substance is a protein compounded with insulin. The most possible protein is anti-insulin antibody for molecular weight of IgG is 150KD. It seems to be more likely that there is another peak of free insulin, but the result of chromatography indicates that all of the insulin molecules in the serum sample are bound to anti-insulin antibodies at room temperature. Hence we can make a diagnosis of this patient hypoglycemia, which is autoimmune hypoglycemia caused by anti-insulin antibody.

Case 2

A 71-year-old male patient diagnosed as Type 2 diabetes mellitus for 18 years and accepted Insulin therapy for about 3 years, was admitted to PUMCH for recurrent ketosis for last 3 months and DKA twice on Sept.^{6th}, 2010, while the total amount of insulin dosage he used per day was 650-800 IU. Meanwhile, the blood glucose concentration could not be controlled yet.

He was diagnosed as type 2 diabetes mellitus in 1992 and began to receive the oral antidiabetic drugs such as metformin and glibenclamide. Then he accepted insulin therapy (Gansulin Mix 30®) due to poor glycemic control in 2007. After that, his fasting serum glucose levels maintained 7-8mmol/l when the total insulin amount used per day was 40IU. However, the patient felt more and more fatigue, accompanied with polyuria, thirsty and polydipsia since spring of 2010, and the

fasting glucose level was as high as 20mmol/l. Although the daily dosage increased to more than 100 IU gradually, the blood glucose control of the patient was not improved at all. On the contrary, he had suffered from recurrent ketosis, and had been diagnosed as diabetic ketoacidosis twice since the end of May, 2010. In the process of treatment of DKA, he was transfused intravenously with insulin and his daily dosage increased to 500-800 IU to prevent the recurrence of DKA but the blood glucose level remained between 12 to 20mmol/L. His weight dropped 27 kg in half a year. And he had a dumb feeling in both feet for about half a year. Finally he was admitted to PUMC Hospital.

On examination, the patient appeared extremely slim, intravenously infused with recombinant human regular insulin by an intravenous pump. His weight was 53kg and his height was 165cm. His blood pressure was 110/68mmHg, and the pulse was 84 beats per minute, and the body temperature was normal. Electrocardiography, echocardiography and abdominal ultrasound were normal. Laboratory data revealed severe hyperglycemia (25mmol/L) and obviously high urinary glucose concentration. HbA1c was 10.7% although the patient suffered from slightly anemia (Hb 112g/L, reference range 120-160g/L). Chest X-ray showed patchy shadows in the right pulmonary lobe and Chest CT revealed multiple patchy shadows in the right middle lobe. PET-CT showed parts of the shadows with high uptake of 18F-FDG.

When the blood glucose attained 25mmol/L and the patient was intravenously infused with recombinant regular human insulin continuously, we drew his blood sample from the lateral forearm and determined the serum insulin and C-peptide level. The result of serum insulin level was 0mIU/L while the serum C-peptide was 2.1mmol/L, which was completely inconsistent.

Hence recovery test was done to test what had happened. After adding insulin solutions by insulin standard substance, saline and bovine serum albumin, which final theoretical concentrations would have been 18.1mmol/L and 33.3mmol/L, respectively, the practical concentrations were determined to calculate either recovery rate. However, both results were 0mmol/L, which meant the recovery rate was 0%. The serum anti-insulin antibody concentration was determined immediately but the exact value could not be acquired because it was more than the upper limit of the reference 0-10mIU/L.

Diagnosis of anti-insulin antibody induced hyperglycemia was determined and the plasma exchange therapy started. But the clinical effect was not satisfactory after using this treatment 3 times (every other day). Hence the immune-suppressive therapy was adopted and he took prednisone 30mg per day and mycophenonate 5mg three times a day. After initiation of immune-suppressive therapy, the total dosage of insulin was dropped gradually and infusing insulin intravenously was substituted by multiple subcutaneous injections, and the patient was discharged.

4 months later, the patient was followed up in outpatient clinic. The total dosage was decreased to 35IU/d and HbA1c was 7.3%. His dosage of prednisone was reduced to 30mg and mycophenonate was 5mg twice a day. Repeated Chest CT revealed that there was no change in multiple patchy shadows in the right middle lobe.

Discussion of diagnosis

Although we could diagnose the patient as severe insulin resistance syndrome only according to his daily insulin dosage, anti-insulin antibody induced hyperglycemia was the most appropriate diagnosis since it revealed the mechanism of the disease and provided the possible treatment.

The first key point of diagnosis was to determine serum insulin level when the patient was intravenously transfused with insulin. According to the regular dogma of endocrinologist, measuring insulin was not permitted when the patient accepted insulin therapy. But the fact that such large dosage of infused insulin could not lower blood glucose level was so wired that we believed serum insulin level would give us some useful information. However, the result that serum insulin level was 0mmol/L was extremely surprising, which meant two possibilities – enzyme which could hydrolyze insulin or high molecular protein which could bind insulin and mask the isotopes of insulin in the patient's blood. Then we did the recovery test.

The recovery test was the second point of diagnosis and it excluded the possibility of enzyme hypothesis. After the freshly concentrated prepared insulin solution was added to the patient's serum in vitro, the insulin level measured was still 0mmol/L. Since activity of enzyme will drop rapidly in the room temperature, the hypothesis of enzyme could be excluded. Meanwhile, the hypothesis of high molecular protein could explain this situation: there should be so large amount of

such protein in the patient's blood that all of the added insulin could be bound. And result of measurement of anti-insulin antibody proved this hypothesis and revealed that anti-insulin antibody was the high molecular protein we were looking for.

Although chest-CT and PET-CT suggested possibilities of malignant tumor, no clear evidence can provide any clues explaining the relation between malignant tumor and anti-insulin antibody. We did not have any evidence of lymphoma, while senior respiratory physician was inclined to the diagnosis of bronchiectasis based on the results of chest-CT. Since PET-CT was performed when the patient had experienced a long period of severe hyperglycemia, the nonspecific shadows with high uptake of 18F-FDG were considered to be result of hyperglycemia without diagnosis value. Follow-up of this patient confirmed our diagnosis.

Finally, we made it clear that anti-insulin antibody band both the infused recombinant human insulin and endogenous insulin so that no insulin in the patient's blood could have any effect, which caused hyperglycemia and its acute complications directly.

Discussion of treatment

Plasma exchange was selected as the first choice because it was believed to clear the serum antibody directly and to improve the clinical symptoms rapidly[7]. However, it didn't acquire the ideal effect after the patient accepted it for a week. Hence we adopted the immuno-suppressive therapy with prednisone and mycophenolate, which was commonly used in autoimmune diseases and was effectively used in some similar cases[7,8]. As a result, the blood glucose was well controlled and the daily insulin dosage decreased dramatically. And follow-up of this patient demonstrated the effectiveness of immune-suppressive therapy.

Discussion of classification of insulin-related antibody induced dysglycemia

These two cases are both induced by anti-insulin antibody, although the clinical investigations are completely opposite. However, based on the same mechanism, the treatments of the two cases are similar. It is reasonable that they should be classified to two subtypes of one disease. Unfortunately, these two cases have to be classified to completely different categories according to their clinical

symptoms: autoimmune hypoglycemia and severe insulin resistance syndrome. Although the current classification is useful for clinical physicians, we still believe that a new classification according to mechanism of disease is also useful to guide treatments and will be more useful in the future (Table 2, Fig. 2). First, we classify insulin-related antibody induced dysglycemia as three categories according to different etiology of immune response. Mechanism of the first category is that insulin antibody induces autoimmune attack to beta cells, which finally develops to type 1 diabetes[9]. The second category is induced by insulin-receptor antibody. This type of antibody always binds on insulin receptor of the surface of target cells. If the antibody belongs to antagonist, it will induce hyperglycemia (often called type B insulin resistance); if the antibody is a kind of agonist, it will induce persistent hypoglycemia, similar as mechanisms of Graves' disease. The third category is induced by circulating antigen-antibody complex, while insulin is the special antigen. Extremely high binding capacity between antibody and insulin will make all the insulin molecules unable to bind with insulin receptor, which will cause severe hyperglycemia such as case 2 in this article. If the binding capacity is not very high while the amount of antibody is large, paroxysmal hypoglycemic attacks will happen when large amounts of insulin molecules are dissolved from antigen-antibody complex. And we also can speculate antigen epitope from the reaction of therapy of insulin analogues, while the current IAS patients can be classified to one subgroup with specific antigen epitope(s). One case was successfully treated by insulin analogue Lispro[10], which is possibly due to avoiding antigen epitope. Hence it will be important in the future to differentiate those subgroups according to antigen epitopes; when most common antigen epitopes of insulin can be identified in clinical assay and various types of insulin analogues can be selected, we can use special insulin analogues in order to avoid specific epitopes in most of those patients.

In this article, we provide two rare cases induced by anti-insulin antibody. Anti-insulin antibody induced hypoglycemia is relatively common, but the case with false negative determination of anti-insulin antibody is rare and difficult. Anti-insulin antibody induced hyperglycemia was extremely rare, and the case with serum insulin level being 0mmol/L has not been reported in literature. The common characteristics of these two cases is not only caused by the same antibody,

Table 2. Classification of insulin-related antibody induced dysglycemia

	Insulin-related antibody induced dysglycemia			
Category	Type 1 diabetes	Insulin-receptor antibody induced dysglycemia	Insulin antibody induced dysglycemia	
Mechanism	Insulin antibody induced autoimmune attack to beta cell	Antibody acts as a legend of insulin receptor	Circulating complex composed by insulin and insulin antibody influences the concentrations of free insulin.	
Antigen	Insulin	Insulin-receptor antibody	Insulin/Insulin analogues	Cross antigen for mercapto Group (Hirata disease)
Antigen epitope			Identified in the future	Near mercapto group of insulin

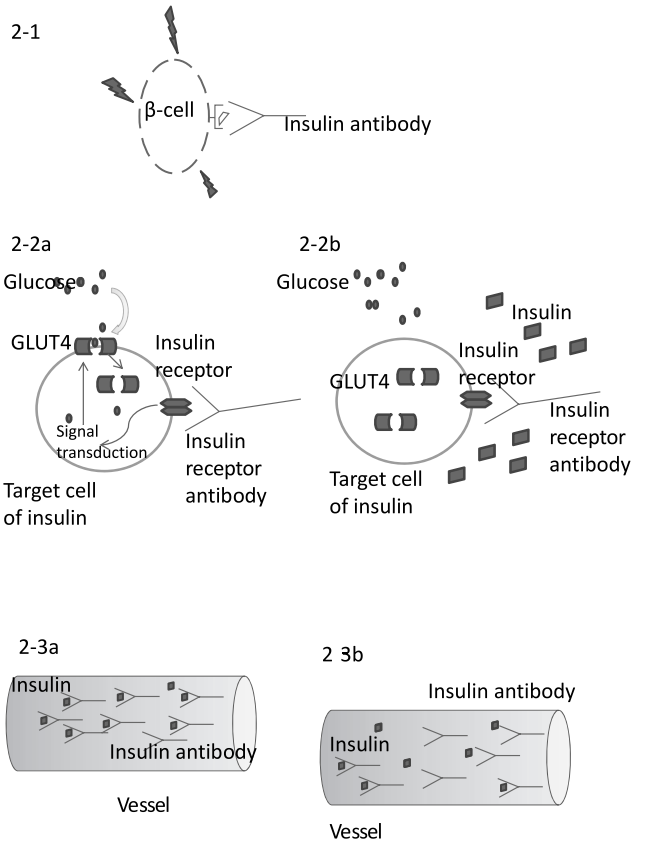

Fig. 2. Three different mechanisms of insulin-related-antibody-mediated dysglycemia. (2-1) Insulin antibody induces β-cell damage through complimentary system, ADCC, etc. This situation often causes type 1 diabetes, following with hyperglycemia. (2-2) Insulin-receptor antibody induces dysglycemia through binding with insulin receptor. When it acts as agonist, the patient will suffer from hypoglycemia (2-2a); when it acts as an antagonist, severe hyperglycemia happens (2-2b). (2-3) Complex of insulin antibody and insulin in circulation causes dysglycemia. If the binding capacity is extremely high, patient will suffer from persistent hyperglycemia (2-3a); if binding capacity is not very high but the concentrations of antibody are high, hypoglycemic attacks will happen frequently (2-3b).

but also diagnosed through the laboratory experiments based on basic medicine. The first case is diagnosed through column chromatography, while the second is by recovery test. Based on mechanism revealed by these two cases, we suggest a new classification of insulin-related antibody induced dysglycemia.

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CHAPTER 6

Polygenic Risk Models Suggest Protective Effects of Lipid Genes on Plasma Glucose, HbA1c and HOMA-IR levels

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Abstract:

OBJECTIVE: Dyslipidemia is strongly associated with raised glucose levels and its clinical endpoint of type 2 diabetes (T2D). However, the causal nature of this relationship and the effect of lipid genes on T2D remain unclear. The identification of lipid SNPs in genome-wide association studies has enabled the use of polygenic risk scores to assess the nature of the relationship between lipids and glucose-related traits.

RESEARCH DESIGN AND METHODS: We used 95 common genetic variants that were robustly associated with triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) or high-density lipoprotein cholesterol (HDL-C). We calculated polygenic scores for blood lipids in 13,105 subjects of the LifeLines cohort and in a replication sample of 3649 subjects from the PREVEND cohort. Then we computed Spearman correlations of each genetic score with fasting plasma glucose, HbA1c levels and HOMA-IR. We further performed meta-analysis to combine the effects of individual lipid SNPs on glucose-related traits in LifeLines and PREVEND using a weighted Z-score approach.

RESULTS: We found no associations between lipid risk scores and glucose-related traits except for a weak negative correlation between the TG risk score and HbA1c (r=-0.025, p=0.01). However, after adjusting for circulating lipid levels, higher TG, LDL and TC risk scores and a lower HDL risk score are correlated with lower levels of glucose-related traits. Specifically, the TG risk score was positively correlated with TG levels (r=0.19, $p=4.2\times10^{-91}$ in LifeLines; r=0.21, $p=4.5\times10^{-25}$ in PREVEND) but negatively correlated with fasting glucose (r=-0.058, p=9.6×10-10 in LifeLines; r=-0.044, p=0.03 in PREVEND), HbA1c (r=-0.048, $p=4.2\times10^{-7}$ in LifeLines) and HOMA-IR (r=-0.07, $p=6.2\times10^{-4}$ in PREVEND). In LifeLines, we also observed similar negative correlations between glucose-related traits and the LDL risk score (r=-0.029, $p=2.6\times10^{-3}$ for fasting glucose; r=-0.035, $p=2.2\times10^{-4}$ for HbA1c) or TC (r=-0.022, p=0.019 for fasting glucose; r=-0.038, $p=5.7\times10^{-5}$ for HbA1c). The HDL risk score was positively correlated with both HDL and fasting glucose levels (r=0.036, $p=1.8\times10^{-4}$ in LifeLines; r=0.063, $p=1.9\times10^{-3}$ in PREVEND), HbA1c (r=0.028, p=0.003 in LifeLines) and HOMA-IR (r=0.113, $p=3.2\times10^{-8}$ in PREVEND). Furthermore, we identified 15 loci at $p<0.01$ level with pleiotropic effects on lipids and glucose-related traits, and 8 of them (APOB, GCKR, TIMD4, MLXIPL, CYP7A1, CETP, APOE-C1-C2 and PLTP) have shown significant associations with these two traits opposite to the expected phenotypic directions.

CONCLUSIONS: Our polygenic risk models for lipids show weak but highly significant protective effects on fasting plasma glucose, HbA1c and HOMA-IR levels. These paradoxical protective effects are caused by pleiotropy as they are independent of the lipid levels.

Introduction

Dyslipidemia, which includes increased circulating concentrations of total triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and/or decreased circulating high-density lipoprotein cholesterol (HDL-C) levels, is known to be strongly associated with insulin resistance (IR) and type 2 diabetes (T2D). This is especially true for hypertriglyceridemia and decreased HDL-C levels as these are important components of the metabolic syndrome.

Current wisdom dictates that the relation between dyslipidemia and IR and/or T2D is explained by a causal effect of IR and/or T2D on dyslipidemia. IR may cause compensatory hyperinsulinemia and high plasma free fatty acids (FFA), which may induce hypersecretion of very-low-density lipoprotein cholesterol (VLDL-C) subsequently leading to dyslipidemia[1-3]. However, in the current study we will explore two often neglected alternative explanations.

The first possibility is that instead of IR and/or T2D causing dyslipidemia the reverse may be true, i.e., dyslipidemia may cause IR, leading to higher levels of glucose and related traits and eventually T2D. This explanation is consistent with evidence from some epidemiological studies. Circulating HDL-C level has been shown to be a predictor of future IR or T2D[4,5], while some lipid-lowering therapy can reduce the incidence of T2D[6,7]. With the identification of over 90 lipid loci in genome-wide association studies (GWASs)[8] a method has emerged that enables testing the direction of causal relationships. The effects of lipid genes are combined in genetic risk scores (GRSs), which can be used as proxies for blood lipid levels to test their causal effect on glucose-related traits through a polygenic model. This is also known as the Mendelian Randomization approach[9]. A similar study was first performed by De Silva et al[10], who detected no significant effects of TG genes on fasting glucose level, IR and risk of T2D concluding that TG is not a causal factor for these traits. Another study by Qi et al[11] adopted a similar design, but found positive effects for TG and HDL-C GRSs indicating these lipids may be causally related to glucose-related traits and outcomes. As the only two previous studies testing this hypothesis reached opposite conclusions, larger studies using GRSs containing more lipid SNPs are warranted.

A third possibility explaining the relation of dyslipidemia with IR and/or T2D

is pleiotropic effects of genes independently influencing both lipids and glucose-related traits. It seems increasingly clear that closely associated phenotypes often share genes and pathways, especially for common complex diseases[12,13]. Results from the GWAS catalog show that 16.9% of genes and 4.9% of SNPs show pleiotropic effects[14]. Also for dyslipidemia and T2D (or other glucose-related traits) one would expect many genes to have pleiotropic effects on these traits. However, only 5 lipid genes (GCKR, FADS1, IRS1, KLF14 and HFE) also showed genome-wide significant effects on T2D or glucose-related traits in spite of GWASs having discovered over 95 loci for dyslipidemia[8] and about 50 loci for T2D[15] in recent years. This is inconsistent with the expectation of abundant pleiotropy of complex diseases and traits. It is still possible that pleiotropic genetic effects of lipid genes on glucose-related traits may be too small to identify in GWASs, which also can be tested by the polygenic model.

We performed the current polygenic risk model analysis to further investigate the relationship between lipid genes and glucose-related traits using the LifeLines and PREVEND studies with data available on both genotype and phenotype in more than 15,000 subjects. The aims (Fig. 1) are: 1). To test whether GRSs of lipid genes are correlated to glucose-related traits (Path A); 2). To test whether lipid genes have pleiotropic effects on glucose-related traits (Path B). Furthermore, we performed single SNP association to test the impact of lipid SNPs on glucose related traits, before or after adjusting for circulating lipid levels.

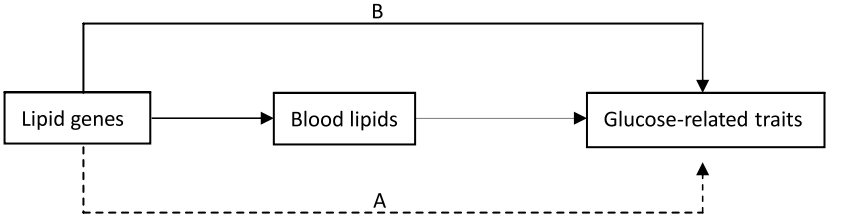

Fig. 1. The possible relation between lipid genes and glucose-related traits (according to principles of Mendelian randomization approch[16]). In this article we investigated the effects of lipid genes on glucose-related traits. The effect could be indirectly mediated through blood lipid levels (Path A); it also could be direct, independent from blood lipid levels (Path B).

RESEARCH DESIGN AND METHODS

Study cohort

The study was approved by the Ethics Committee of the University Medical Centre Groningen.

The LifeLines cohort. In this study we used 13,105 genotyped unrelated subjects derived from the LifeLines cohort study, a large cohort study of representative Caucasian residents in three Northern provinces of the Netherlands which will include 165,000 participants[17]. All participants underwent a medical examination at baseline since the end of 2006 and will be followed for 30 years. This study used the clinical measures at the baseline. TC was measured with an enzymatic colorimetric method, HDL-cholesterol with a colorimetric method, and TG with a colorimetric UV method. Fasting plasma glucose (FPG) was measured with a hexokinase method. The HbA1c level was measured using a turbidimetric inhibition immunoassay but standardized against the reference method of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The LDL cholesterol concentration was calculated using the Friedewald equation. More details were described in the previous paper[18,19]. Fasting insulin was not measured in the LifeLines cohort preventing calculation of HOMA-IR.

The PREVEND cohort. We used a subset of 3,649 genotyped unrelated subjects from the Prevention of REnal and Vascular ENd stage Disease (PREVEND) study, which is an ongoing prospective study since 1997 investigating the natural course of increased levels of urinary albumin excretion and its relation to renal and cardiovascular disease. The initial cohort consisted of 8,592 subjects. This study used the clinical measures at baseline. HDL cholesterol was measured with a homogeneous method (direct HDL, AEROSETTM System, Abbott Laboratories, Abbott Park, U.S.A.). Triglycerides were measured on a Mega multi-analyzer after enzymatic splitting with lipoprotein lipase (GPO PAP, Merck, Darmstadt, Germany). Total cholesterol and plasma glucose were assessed using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York, U.S.A.). Insulin was measured with an AxSym® auto-analyzer (Abbott Diagnostics, Amstelveen, The Netherlands). The details of the protocol for measuring HDL, TG, TC, FG and insulin levels have been described before[20,21]. The LDL cholesterol concentration

was calculated using the Friedewald equation. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by (glucose×insulin)/22.5. The HbA1c level was not measured in PREVEND cohort.

The selection of subjects

This study we only included the clinic measures of fasting blood samples from non-diabetes individuals. Diabetes was defined by any one of the following criteria if applicable: 1) self-reported diabetes; 2) FPG ≥7.0 mmol/L; 3) HbA1c ≥6.5%; or 4) taking anti-diabetic medicines. We also excluded the individuals taking lipid-lowering medicines. The participants should be fasting for 8 hours before clinic measurements. We did not exclude the individuals with anemia, as we did not observe any significant impact of anemia on HbA1c level (T-test P value 0.44). Thus the final study population comprised 10,995 LifeLines subjects and 2,438 PREVEND subjects. The clinical characteristics of the cohort are summarized in Table 1.

Table 1: Clinical characteristics of Cohorts

	LifeLines	PREVEND
Number of Individuals	10,995	2,438
Covariates		
Sex (Male/Female)	4,441/6,554	1158/1280
Age (mean + s.d.)	47.5 + 10.7	49.8 +12.5
BMI (mean + s.d.)	26.1 + 4.1	26.1 + 4.2
Lipids (mean + s.d.)		
HDL-C (mmol/l)	1.46 + 0.39	1.33 + 0.40
LDL-C (mmol/l)	3.34 + 0.88	4.06 + 1.09
TC (mmol/l)	5.16 + 0.98	5.67 + 1.07
Log10(TG)	0.03 + 0.22	0.08 + 0.23
Glucose-related traits (mean +s.d.)		
FPG* (mmol/l)	4.96 + 0.48	4.77 + 0.62
HbA1c (%)	5.50 + 0.31	NA
Log10(HOMA-IR†)	NA	0.23 + 0.28

Genotyping, imputation and quality control.

Both LifeLines and PREVEND cohorts were genotyped using Illumina HumanCytoSNP-12 BeadChip. The un-genotyped SNPs were imputed by program Beagle using HapMap 2 release 24 as the reference panel. We used the best guess imputed genotype for this study. The quality control was described before[18].

SNP selection

A list of lipid SNPs on 95 unique loci was reported from the largest meta-analysis up to date[8]; different lipids can have different best SNPs in the same locus. The risk alleles and their effect size were extracted for each SNP and each lipid type. We further excluded three SNPs that genotypes were not imputed in LifeLines and PREVEND. They are rs13238203 at TYW1B locus; rs2412710 at CAPN3 and rs1800961 at HNF4A. Thus we included 129 lipid-associated SNPs into this study, including 46 SNPs for HDL cholesterol (Sup Table 1), 37 SNPs for LDL cholesterol (Sup Table 2), 30 SNPs for TG (Sup Table 3) and 51 SNPs for TC (Sup Table 4).

Risk score calculation

For each lipid and for each individual, the un-weighted risk score and weighted risk score were calculated respectively. The un-weighted risk score was calculated as the total number of the risk alleles per individual. For the weighted risk score, the risk alleles were weighted by their estimated effect size. We further re-scaled the risk scores between 0 and 1 by dividing the risk scores by the maximum risk. Thus the un-weighted risk model can be described as $S_j = \sum_{j=1}^{j=n} g_{ij} \Big/ 2n$; where g_{ij} is the number of risk alleles for jth SNP in ith individual, coding as 0 for homozygous non-risk alleles, 1 for heterozygous genotype or 2 for homozygous risk alleles; n is the total number of associated SNPs per lipid trait. The weighted risk score is described as $WS_j = \sum_{j=1}^{j=n} \beta_j * g_{ij} \Big/ \sum_{j=1}^{j=n} 2 * \beta_j$; where β_j refers to the estimated effect size for the jth SNP.

Association between the lipid risk score and phenotypes

The TG levels and HOMA-IR level were log^{10} transformed. The residual lipid levels and glucose-related traits were obtained using linear regression model adjusting for covariates: age, age^2, and sex. The model is described as $y_i = age_i +$

$age_i^2 + sex_i + e_i$; where y_i refers to an observed lipid level or glucose related trait (FG, HBA1C or HOMA-IR) for ith individual and e_i is the remaining residual. The residuals were used as phenotypic trait and subject to the Spearman correlation analysis with the lipid risk scores. To tested the potential pleotropic effect of lipid genes on glucose-related traits, we further regressed out the effect of lipids on glucose-related traits using the model $y_i = age_i + age_i^2 + sex_i + HDL_i + LDL_i + TG_i + TC_i + e'_i$; where yi refers to glucose-related trait (FG, HbA1c or HOMA-IR) of ith individual and e' refers to the residuals for glucose traits that are independent from lipid levels. The residuals were used as lipid-independent glucose traits and subject to Spearman correlation with lipid risk scores.

Single SNP association

To test the association between glucose-related traits and individual lipid SNPs, we performed linear regression analysis between the SNP genotype and each phenotypic trait. We further performed meta-analysis to combine the effect of LifeLines and PREVEND using a weighted Z-score approach. The significance of individual SNP was controlled at $P<0.01$.

Result

Observed association between lipid risk scores and lipid levels.

We observed the significant positive correlation between lipid risk scores and the lipid levels observed in our cohorts (Supplementary Table 5, Fig. 2). The weighted risk scores outperformed the un-weighted risk score, explaining substantial proportion of variation in lipid levels: 4.95% for HDL, 3.61% for LDL, 3.61% for TG and 4.41% for TC in LifeLines; 3.69% for HDL, 1.90% LDL; 4.37% TG and 2.07% for TC in PREVEND cohort. It suggests the weighted risk score is a better genetic predictor for the lipid levels. Thus we used weighted risk score for the further analysis. The single SNP association per lipid and the meta-analysis across Lifelines and PREVEND cohorts was shown in Supplementary Table 2. Based on this meta-analysis at P value 0.01 level, we replicated the association for 21 HDL SNPs, 17 LDL SNPs, 12 TG SNPs and 21 TC SNPs (Supplementary Table 1-4).

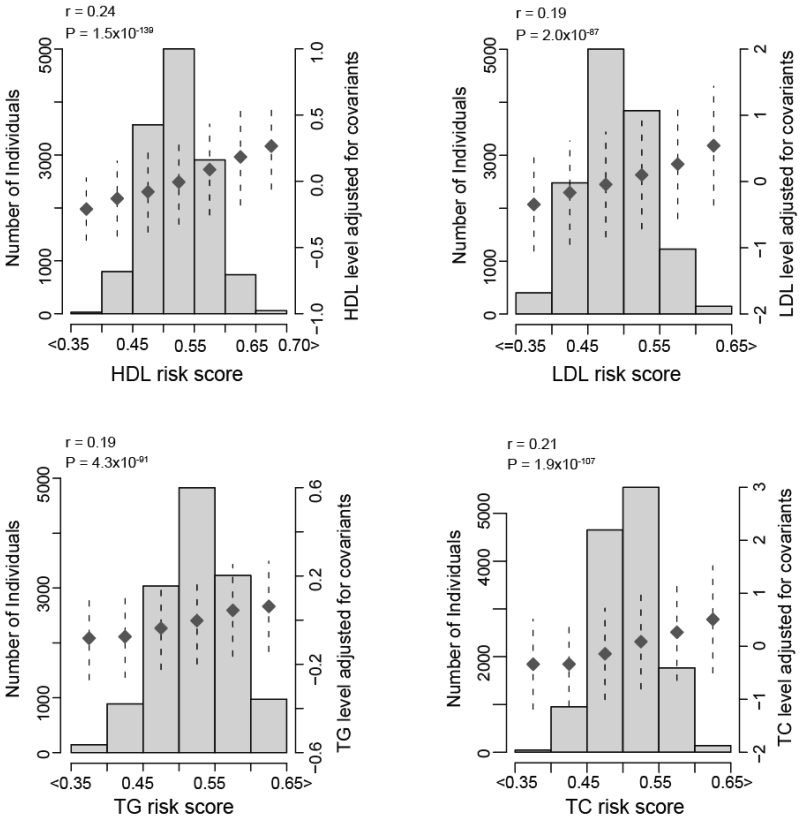

Figure 2: The relationship between lipids and lipid risk scores in Lifelines.

No association between lipid risk scores and glucose-related traits except a weak negative correlation between triglyceride risk score and HbA1c.

We assessed three different glucose-related traits in two independent cohorts: fasting glucose from both LifeLines and PREVEND cohort, HbA1c from LifeLines and HOMA-IR from PREVEND. After adjusting for the covariates, age, age^2 and sex, we observed significant correlation between glucose-related traits and the lipids levels. Consistently with epidemiological observations, individuals who have higher level of LDL, TG and TC levels or have lower HDL levels tend to have higher level of glucose-related traits (Table 2). The strongest correlations were mostly observed for TG. However, despite the strong correlation at phenotypic level, we did not observe the correlation between lipid risk score and glucose-related traits, except a weak negative correlation between triglyceride risk score and HbA1c level in LifeLines (r = - 0.025, P = 0.01) (Sup Table 6).

Table 2: The phenotypic correlation between lipids and glucose-related traits

	HDL (mmol/L)	LDL (mmol/L)	Log_{10}(TG)	TC (mmol/L)
LifeLines				
FPG (mmol/l)	r = -0.159 p = 5.1×10^{-63}	r = 0.10 p = 2.4×10^{-26}	r = 0.19 p = 1.7×10^{-90}	r = 0.08 p = 4.7×10^{-16}
HbA1c(%)	r = -0.10 p = 6.1×10^{-25}	r = 0.10 p = 2.4×10^{-26}	r = 0.19 p = 1.7×10^{-90}	r = 0.11 p = 8.1×10^{-33}
PREVEND				
FPG (mmol/l)	r = -0.17 p = 6.0×10^{-17}	r = 0.12 p = 5.8×10^{-9}	r = 0.15 p = 5.6×10^{-13}	r = 0.09 p = 1.8×10^{-5}
Log10(HOMA-IR)	r = -0.34 p = 7.5×10^{-66}	r = 0.21 p = 5.8×10^{-25}	r = 0.43 p = 1.2×10^{-109}	r = 0.15 p = 8.0×10^{-14}

Pleotropic association between lipid risk scores and glucose-related traits.

We further regress out the phenotypic correlation structure between lipids and glucose-related traits and obtained lipid-independent glucose traits. To our surprise, we observed, at genetic level, an opposite effect between dyslipidemia and glucose-related traits: higher TG, LDL and TC risk score or lower HDL risk score were correlated with lower glucose-related traits (Table 3). Specifically, triglyceride risk score were positively correlated with triglyceride level ($p=4.2\times10^{-91}$ in LifeLines and $p=4.5\times10^{-25}$ in PREVEND) but negatively correlated with fasting glucose ($p=9.6\times10^{-10}$ in LifeLines, p=0.03 in PREVEND), HbA1c ($p=4.2\times10^{-7}$ in LifeLines) and HOMA-IR ($p=6.2\times10^{-4}$ in PREVEND). In LifeLines, we also observed the similar negative correlation between glucose-related traits and the risk scores of LDL or TC. Contrary to other lipid types, HDL risk score were positively correlated with HDL level and also positively correlated with fasting glucose level ($p=1.8\times10^{-4}$ in LifeLines and $p=1.9\times10^{-3}$ in PREVEND), HbA1c (P=0.003 in LifeLines) and HOMA-IR ($P=3.2\times10^{-8}$ in PREVEND). Our analysis suggests that the negative genetic effect is overlooked due to the strong phenotypic correlations in the opposite direction.

Single SNP association

At P value 0.01 level, we detected the association at 18 lipid SNPs on 15 unique loci with lipid-independent glucose-related traits: 11 SNPs for fasting

Table 3: The Spearman correlation between lipid risk score and lipid-independent glucose trait

LifeLines		
	FPG (mmol/l)	HbA1c (%)
HDL risk scores	r = 0.036 p = 1.8×10^{-4}	r = 0.028 p = 0.003
LDL risk scores	r = -0.029 p = 2.6×10^{-3}	r = -0.035 p = 2.2×10^{-4}
TG risk scores	r = -0.058 p = 9.6×10^{-10}	r = -0.048 p = 4.2×10^{-7}
TC risk scores	r = -0.022 p = 0.019	r = -0.038 p = 5.7×10^{-5}
PREVEND		
	FPG (mmol/l)	HOMA
HDL risk scores	r = 0.063 p = 1.9×10^{-3}	r = 0.113 p = 3.2×10^{-8}
LDL risk scores	r = 0.028 p = 0.17	r = -0.003 p = 0.88
TG risk scores	r = -0.044 p = 0.030	r = -0.07 p = 6.2×10^{-4}
TC risk scores	r = 0.018 p = 0.38	r = 0.001 p = 0.96

glucose level, 8 lipid SNPs for HbA1c and 9 SNPs for HOMA-IR (Figure 3 & Sup Table 7). Consistent with the findings of polygenic risk score, 8 of the 15 SNPs (APOB, GCKR, TIMD4, MLXIPL, CYP7A1, CETP, APOE-C1-C2 and PLTP) showed effect directions opposite to the expected phenotypic direction of association between glucose-related traits and lipids. We also compared the allelic direction of lipid SNPs on glucose-related traits before and after adjusting for lipids. Their directions were consistent but the associations became stronger after adjusting for lipid levels (Sup Table 8). This shows that our results are unlikely to be explained by some artifact induced by correcting for phenotypic correlation structure.

Discussion

In the current study, we investigated the relationship between lipid genes and 3 glucose-related traits by polygenic risk model analyses in the large LifeLines

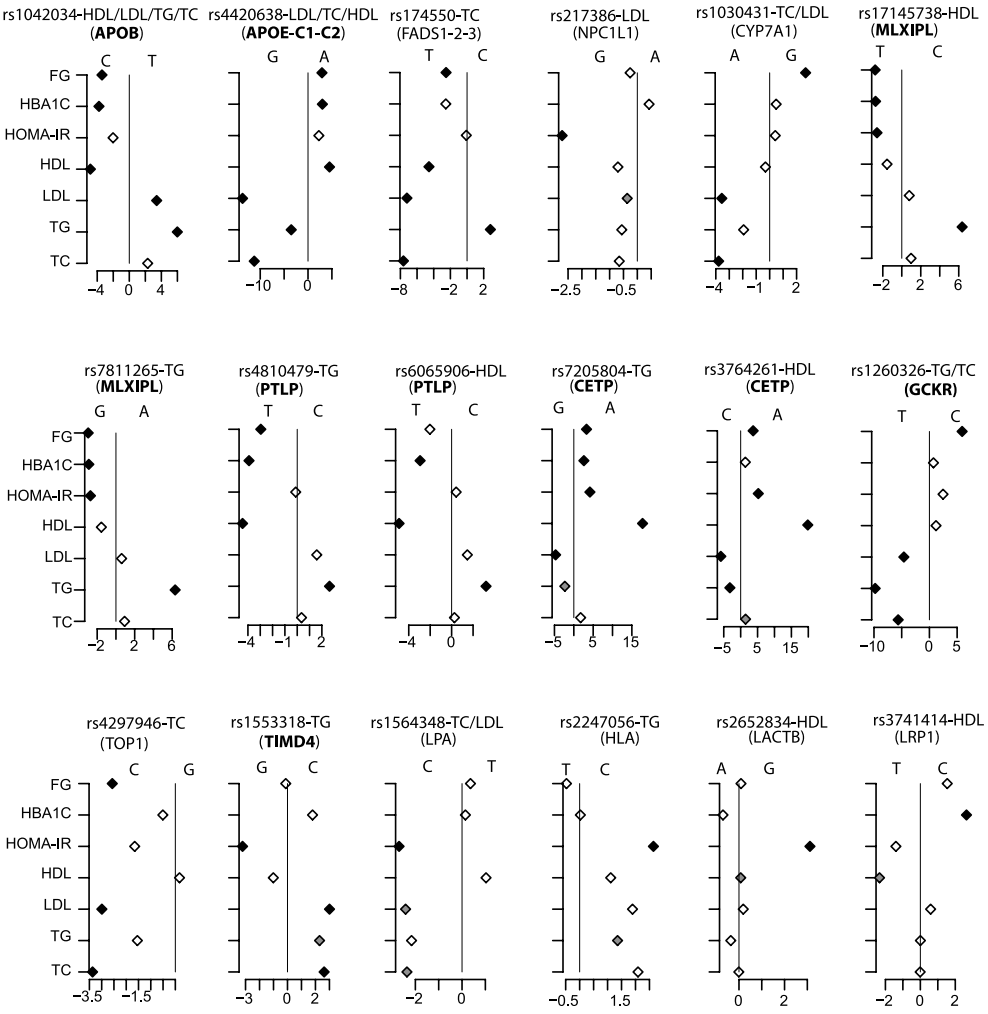

Figure 3: The allelic direction Comparison

X-axis: Z-score of meta-analysis combining the effect of LifeLines and PREVEND. Black dots: significant at P<0.01 level both in our database and in the literatures. Gray dots: significant in the literatures. Open dot: not significant. Bold: genes with opposite effects on lipid and glucose-related traits.

database with replication in PREVEND. First, we examined the relationship between genetic risk scores for each lipid trait and FPG, HbA1c or HOMA-IR (path A in Fig. 1), but found no significant associations except for a weak inverse correlation between the TG genetic score and HbA1c. These results do not support the hypothesis that dyslipidemia has a causal effect on glucose-related traits. These findings are in line with those from a previous study by De Silva et al.[10], as in fact they also found an inverse relationship between the TG genetic score and risk of

T2D or FPG, which is difficult to explain. However, a similar study performed by Qi et al. obtained contradictory results showing that genetic scores of TG or HDL-C can predict risk of T2D[11]. These inconsistent findings based on studies with similar designs have so far failed to improve our insight into the relationship between lipids and glucose traits. Hence we tested an alternative explanation of the relation between lipids and glucose-related traits through pleiotropic effect of lipid genes (Path B in Fig. 1). Unexpectedly, we observed significant associations between genetic risk scores of each type of lipid genes and glucose-related traits after adjusting for circulating lipid levels with directions opposite to the expected phenotypic relationships. This means that alleles increasing TG, TC or LDL-C and alleles decreasing HDL-C have protective (i.e., lowering) effects on glucose-related traits. Furthermore, we detected 18 individual SNPs at 15 loci significantly correlated to at least one lipid - glucose trait combination.

Of the 15 loci (APOB, GCKR, TIMD4, LPA, HLA-B, MLXIPL, NPC1L1, CYP7A1, FADS1-2-3, LRP1, LACTB, CETP, APOE-C1-C2, TOP1 and PLTP) with pleiotropic effects on lipids and glucose-related traits, only GCKR and FADS1 had been reported in previous GWASs to have effects on both traits. We did not confirm the pleiotropic effects of INS1, KLF14 and HFE in our database. Although all are associated with plasma lipid levels, their functions in lipid metabolism vary and have not been completely elucidated. We classified these genes into four categories. First, some of those genes encode plasma proteins closely associated with lipoproteins, including APOB and APOE-C1-C2. Second, some genes encode key enzymes or other functional proteins in lipid metabolism, including MLXIPL, FADS1-2-3, NPC1L1, CYP7A1, CETP and PLTP. MLXIPL is also named ChREBP and encodes a glucose-responsive transcription factor that binds to the promoter of several lipogenic genes. The third category includes GCKR, which encodes glucokinase regulatory protein (GKRP), a protein inhibiting the activity of glycokinase, a key enzyme of glycolysis. Some evidence suggests that activity of glycokinase will influence the amount of the substrate of de novo lipogenesis affecting the blood lipid profile[22]. Other genes including TIMD4, HLA-B, LRP1, LACTB, LPA and TOP1, constitute the fourth category and are involved in a variety of physiological processes. TIMD4 and HLA-B are both associated with immune-related disorders, and LRP1 encodes an endocytic receptor involved in

many physiological processes, including lipid homeostasis. LACTB encodes a protein component of ribosome while TOP1 encodes a DNA topoisomerase for transcription. The pleiotropic effects of those genes may be due to more common physiological processes.

The most surprising results of the current study are that higher genetic risk scores of TG, TC and LDL-C and lower genetic risk scores of HDL-C predict lower FPG, HbA1c and HOMA-IR. In the discovered 15 shared loci associated with both lipids and glucose-related traits, 8 loci (APOB, GCKR, TIMD4, MLXIPL, CYP7A1, CETP, APOE-C1-C2 and PLTP) show significant associations with these two traits opposite to the expected phenotypic directions. Although this situation is paradoxical, more and more genes are being found to have similar pleiotropic effects, such as PNPLA3 for TG and T2D in obese individuals[23], GRB14 for WHR and FPG, SLC39A8 for BMI and HDL-C[24]. However, its significance remains unclear. GCKR was the first gene for which opposite effects on lipid and glucose-related traits were reported[25]. When the glucose-lowering allele reduces the activity of GKRP, the activity of GCK will increase and de novo lipogenesis will speed up, which eventually causes increased synthesis of TG. Furthermore, TG in hepatocytes will induce lipotoxicity and hypertriglycemia, which may induce IR and down-regulate the activity of GCK, thus acting as a “negative feedback system”. However, GCKR is more a glucose gene than a lipid gene. It remains to be seen how pleiotropic effects of the other genes can be explained. From an evolutionary perspective, all of the 8 genes are highly conserved, which suggest that opposite pleiotropic effects may be common in evolution[26,27].

In conclusion, we tested the effects of lipid genes on glucose-related traits by polygenic model analyses, and found that lipid genes have pleiotropic effects on fasting plasma glucose levels, HbA1c and HOMA-IR. The most notable finding is that these pleiotropic effects are opposite to the directions of the phenotypic associations, which means that individuals with more alleles increasing TG, TC and LDL-C and decreasing HDL-C would have lower plasma glucose levels, HbA1c and HOMA-IR. We also detected 15 gene loci having pleiotropic effects, and 8 of them were revealed to produce opposite effects on blood lipids and glucose-related traits. Further research investigating the underlying mechanisms of these pleiotropic effects is warranted.

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Supplementary Table 1: The HDL SNPs and their association in LifeLines and PREVEND

HDL SNPs						LifeLines			PREVEND			Meta
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE§)	P value	RAF	beta (SE)	P value	P value
16	CETP	rs3764261	55,550,825	A	0.087801	0.32	0.095 (0.005)	3.68×10^{-79}	0.33	0.073 (0.011)	1.88×10^{-11}	6.06×10^{-87}
8	LPL	rs12678919	19,888,502	G	0.058275	0.1	0.064 (0.008)	9.17×10^{-17}	0.1	0.064 (0.017)	2.08×10^{-4}	1.04×10^{-18}
18	LIPG	rs7241918	45,414,951	T	0.033929	0.84	0.039 (0.006)	1.79×10^{-09}	0.85	0.011 (0.014)	0.438	2.38×10^{-9}
20	PLTP	rs6065906	43,987,422	T	0.024087	0.8	0.026 (0.006)	1.30×10^{-5}	0.81	0.041 (0.014)	2.82×10^{-3}	1.20×10^{-6}
2	APOB	rs1042034	21,078,786	C	0.02331	0.21	0.026 (0.006)	8.14×10^{-6}	0.21	0.029 (0.013)	0.0213	1.53×10^{-6}
19	APOE–C1–C2	rs4420638	50,114,786	A	0.027454	0.86	0.03 (0.007)	3.91×10^{-5}	0.86	0.034 (0.015)	0.0255	8.04×10^{-6}
1	PABPC4	rs4660293	39,800,767	A	0.012432	0.75	0.023 (0.005)	1.76×10^{-5}	0.74	0.013 (0.012)	0.291	1.14×10^{-5}
11	LRP4	rs3136441	46,699,823	C	0.020202	0.15	0.028 (0.007)	3.54×10^{-5}	0.15	0.023 (0.015)	0.125	1.43×10^{-5}
8	PPP1R3B	rs9987289	9,220,768	G	0.031339	0.93	0.033 (0.009)	2.73×10^{-4}	0.92	-0.018 (0.019)	0.347	7.29×10^{-4}
11	FADS1–2–3	rs174601	61,379,716	C	0.018907	0.78	0.02 (0.006)	8.25×10^{-4}	0.78	0.003 (0.013)	0.813	8.16×10^{-4}
9	TTC39B	rs643531	15,286,034	A	0.018648	0.84	0.023 (0.006)	4.65×10^{-4}	0.85	-0.007 (0.014)	0.637	8.19×10^{-4}
4	SLC39A8	rs13107325	103,407,732	C	0.021756	0.93	0.027 (0.009)	4.35×10^{-3}	0.93	0.029 (0.021)	0.161	1.7×10^{-3}
16	CMIP	rs2925979	80,092,291	C	0.011655	0.65	0.015 (0.005)	4.47×10^{-3}	0.65	0.011 (0.011)	0.315	2.26×10^{-3}
1	ZNF648	rs1689800	180,435,508	A	0.012173	0.68	0.015 (0.005)	4.79×10^{-3}	0.68	0.009 (0.011)	0.428	2.76×10^{-3}
2	IRS1	rs1515100	226,837,161	C	0.011914	0.54	0.016 (0.005)	2.44×10^{-3}	0.53	-0.002 (0.011)	0.868	2.79×10^{-3}
8	TRIB1	rs10808546	126,565,000	T	0.015799	0.46	0.013 (0.005)	6.99×10^{-3}	0.45	0.012 (0.01)	0.242	3.09×10^{-3}
12	SCARB1	rs838880	123,827,546	C	0.015799	0.35	0.012 (0.005)	0.0179	0.34	0.022 (0.011)	0.0430	4.56×10^{-3}

to be continued

continued

HDL SNPs						LifeLines			PREVEND			Meta
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE§)	P value	RAF	beta (SE)	P value	P value
22	UBE2L3	rs181362	20,262,068	C	0.011914	0.78	0.015 (0.006)	9.84×10^{-3}	0.79	0.012 (0.013)	0.367	5.00×10^{-3}
7	KLF14	rs4731702	130,083,924	T	0.015281	0.49	0.013 (0.005)	5.98×10^{-3}	0.49	-0.005 (0.01)	0.664	6.98×10^{-3}
15	LIPC	rs1532085	56,470,658	A	0.037555	0.3	0.015 (0.006)	0.0104	0.31	0.005 (0.013)	0.723	7.18×10^{-3}
17	STARD3	rs881844	35,063,744	G	0.013209	0.72	0.012 (0.005)	0.031	0.7	0.019 (0.012)	0.0975	9.58×10^{-3}
17	ABCA8	rs4148008	64,386,889	C	0.010878	0.68	0.011 (0.005)	0.0291	0.68	0.016 (0.011)	0.173	0.0105
12	LRP1	rs3741414	56,130,316	T	0.011914	0.24	0.01 (0.006)	0.0676	0.24	0.031 (0.012)	0.0106	0.013
11	UBASH3B	rs7115089	122,035,801	G	0.008029	0.37	0.01 (0.005)	0.0366	0.35	0.009 (0.011)	0.396	0.0168
12	SBNO1	rs4759375	122,362,191	T	0.022274	0.06	0.017 (0.01)	0.0923	0.06	0.025 (0.023)	0.272	0.034
12	ZNF664	rs4765127	123,026,120	T	0.011396	0.31	0.01 (0.005)	0.0516	0.33	-0.002 (0.011)	0.859	0.0352
8	TRPS1	rs2293889	116,668,374	G	0.011396	0.58	0.007 (0.005)	0.132	0.58	0.019 (0.011)	0.0758	0.0358
19	ANGPTL4	rs7255436	8,339,196	A	0.011655	0.55	0.007 (0.005)	0.127	0.55	0.014 (0.01)	0.168	0.0402
12	MVK	rs7134594	108,484,576	T	0.011396	0.52	0.007 (0.005)	0.151	0.49	0.013 (0.01)	0.232	0.0501
2	COBLL1	rs12328675	165,249,046	C	0.017612	0.12	0.011 (0.007)	0.115	0.13	0.008 (0.016)	0.615	0.0514
5	ARL15	rs6450176	53,333,782	G	0.012691	0.74	0.009 (0.005)	0.103	0.73	0.002 (0.012)	0.868	0.053
19	LILRA3	rs386000	59,484,573	C	0.021497	0.01	0.03 (0.021)	0.153	0.02	0.032 (0.043)	0.454	0.0594
7	MLXIPL	rs17145738	72,620,810	T	0.014763	0.13	0.01 (0.007)	0.141	0.12	0.009 (0.016)	0.590	0.0597
6	LPA	rs1084651	161,009,807	G	0.014504	0.85	0.008 (0.007)	0.223	0.85	0.017 (0.015)	0.230	0.0698

to be continued

continued

HDL SNPs						LifeLines			PREVEND			Meta
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE§)	P value	RAF	beta (SE)	P value	P value
12	PDE3A	rs7134375	20,365,025	A	0.01036	0.4	0.008 (0.006)	0.174	0.41	-0.009 (0.013)	0.488	0.0997
18	MC4R	rs12967135	56,000,003	G	0.010878	0.75	0.006 (0.005)	0.279	0.76	0.003 (0.012)	0.808	0.108
6	CITED2	rs605066	139,871,359	T	0.010101	0.55	0.01 (0.01)	0.314	0.53	0.014 (0.025)	0.578	0.108
9	ABCA1	rs1883025	106,704,122	C	0.024346	0.75	0.006 (0.006)	0.367	0.74	0.011 (0.013)	0.429	0.115
17	PGS1	rs4082919	73,889,077	T	0.01036	0.52	0.003 (0.005)	0.542	0.51	0.018 (0.011)	0.0810	0.124
11	APOA1–C3–A4–A5	rs964184	116,154,127	C	0.03885	0.98	0.015 (0.016)	0.374	0.98	0.001 (0.036)	0.974	0.136
11	AMPD3	rs2923084	10,345,358	A	0.010619	0.83	-0.004 (0.006)	0.524	0.84	-0.011 (0.014)	0.457	0.147
16	LCAT	rs16942887	66,485,543	A	0.032893	0.02	0.008 (0.019)	0.665	0.01	0.077 (0.05)	0.124	0.15
1	GALNT2	rs4846914	228,362,314	A	0.015799	0.62	0.003 (0.007)	0.677	0.59	0.015 (0.011)	0.160	0.155
6	C6orf106	rs2814944	34,660,775	G	0.012691	0.93	0.004 (0.009)	0.66	0.94	0.007 (0.022)	0.751	0.176
19	LOC55908	rs737337	11,208,493	T	0.016576	0.999	0.045 (0.068)	0.509	0.999	-0.192 (0.147)	0.193	0.187
15	LACTB	rs2652834	61,183,920	G	0.010101	0.84	-0.001 (0.007)	0.862	0.86	0.018 (0.016)	0.254	0.199

[§]Effect sizes and standard errors (SE) are in s.d. units.

Supplementary Table 2: The LDL SNPs and their association in LifeLines and PREVEND

LDL SNPs						LifeLines			PREVEND			META
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE)	P value	RAF	beta (SE)	P value	P value
19	APOE–C1–C2	rs4420638	50,114,786	G	0.184926	0.14	0.222 (0.017)	6.27×10^{-40}	0.14	0.158 (0.043)	2.77×10^{-4}	3.55×10^{-42}
19	LDLR	rs6511720	11,063,306	G	0.181041	0.92	0.174 (0.021)	9.28×10^{-17}	0.92	0.201 (0.057)	4.04×10^{-4}	1.47×10^{-18}
5	HMGCR	rs12916	74,692,295	C	0.063455	0.39	0.068 (0.011)	2.89×10^{-9}	0.41	0.072 (0.03)	0.0177	4.50×10^{-10}
2	APOB	rs1367117	21,117,405	A	0.104895	0.32	0.062 (0.012)	1.67×10^{-7}	0.32	0.052 (0.031)	0.0980	6.47×10^{-08}
1	LDLRAP1	rs12027135	25,648,320	T	0.02849	0.56	0.058 (0.011)	1.92×10^{-7}	0.56	0.015 (0.029)	0.600	2.72×10^{-7}
1	ANGPTL3	rs3850634	62,823,186	T	0.041181	0.65	0.056 (0.012)	9.90×10^{-7}	0.65	0.053 (0.031)	0.0916	3.60×10^{-7}
1	SORT1	rs629301	109,619,829	T	0.146335	0.71	0.066 (0.015)	1.63×10^{-5}	0.72	0.119 (0.04)	3.030×10^{-3}	1.55×10^{-6}
20	TOP1	rs909802	39,370,229	T	0.036519	0.43	0.038 (0.011)	6.19×10^{-4}	0.44	0.065 (0.03)	0.0292	1.38×10^{-4}
1	IRF2BP2	rs514230	232,925,220	T	0.029267	0.54	0.034 (0.011)	1.78×10^{-3}	0.53	0.077 (0.029)	7.75×10^{-3}	2.77×10^{-4}
8	CYP7A1	rs1030431	59,474,251	A	0.024605	0.33	0.039 (0.012)	8.64×10^{-4}	0.35	0.042 (0.03)	0.162	3.59×10^{-4}
5	TIMD4	rs6882076	156,322,875	C	0.043253	0.65	0.035 (0.012)	2.5×10^{-3}	0.75	0.093 (0.034)	6.68×10^{-3}	$3.\ 80\times10^{-4}$
1	PCSK9	rs2479409	55,277,238	G	0.052059	0.26	0.042 (0.013)	1.14×10^{-3}	0.25	0.042 (0.035)	0.229	5.43×10^{-4}
8	TRIB1	rs2954022	126,551,803	C	0.047656	0.52	0.035 (0.011)	1.5×10^{-3}	0.53	0.037 (0.029)	0.197	6.62×10^{-4}
8	PLEC1	rs11136341	145,115,531	G	0.03626	0.34	0.038 (0.012)	9.31×10^{-4}	0.35	-0.006 (0.031)	0.859	1.22×10^{-3}
11	FADS1–2–3	rs174583	61,366,326	C	0.044289	0.79	0.046 (0.014)	1.26×10^{-3}	0.78	-0.045 (0.038)	0.232	3.07×10^{-3}
2	ABCG5/8	rs4299376	43,926,080	G	0.071225	0.12	0.06 (0.018)	8.9×10^{-4}	0.13	-0.078 (0.047)	0.0969	3.11×10^{-3}
1	MOSC1	rs2807834	219,037,216	G	0.028231	0.78	0.043 (0.016)	5.32×10^{-3}	0.75	-0.043 (0.041)	0.296	8.88×10^{-3}
6	LPA	rs1564348	160,498,850	C	0.050505	0.07	0.045 (0.022)	0.0421	0.07	0.119 (0.059)	0.0446	0.0107
12	BRAP	rs11065987	110,556,807	A	0.025123	0.57	0.022 (0.011)	0.0569	0.56	0.019 (0.031)	0.535	0.0274
16	HPR	rs2000999	70,665,594	A	0.0518	0.41	0.022 (0.012)	0.0713	0.4	0.027 (0.032)	0.394	0.0301

to be continued

continued

LDL SNPs						LifeLines			PREVEND			META
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE)	P value	RAF	beta (SE)	P value	P value
6	FRK	rs11153594	116,461,284	C	0.023051	0.63	0.021 (0.011)	0.0655	0.63	-0.002 (0.031)	0.938	0.0408
10	GPAM	rs1129555	113,900,711	A	0.027972	0.27	0.018 (0.012)	0.154	0.28	0.046 (0.033)	0.170	0.048
6	MYLIP	rs3757354	16,235,386	C	0.037037	0.78	0.022 (0.013)	0.106	0.78	0.018 (0.036)	0.620	0.0481
11	ST3GAL4	rs11220462	125,749,162	A	0.050505	0.08	0.032 (0.02)	0.109	0.08	0.021 (0.055)	0.698	0.0512
6	HFE	rs1800562	26,201,120	G	0.057498	0.99	0.07 (0.061)	0.251	1	0.143 (0.509)	0.778	0.0992
9	ABO	rs649129	135,144,125	T	0.053095	0.08	0.021 (0.021)	0.329	0.07	0.009 (0.062)	0.883	0.123
11	APOA1–C3–A4–A5	rs964184	116,154,127	G	0.073815	0.02	0.039 (0.038)	0.308	0.02	-0.065 (0.103)	0.530	0.138
16	CETP	rs247616	55,547,091	C	0.037555	0.58	0.011 (0.021)	0.585	0.57	0.067 (0.057)	0.240	0.146
12	HNF1A	rs1169288	119,901,033	C	0.036778	0.33	0.008 (0.013)	0.503	0.31	0.009 (0.034)	0.790	0.155
19	CILP2	rs10401969	19,268,718	T	0.080549	1	-0.057 (0.088)	0.521	1	-0.025 (0.247)	0.920	0.162
6	HLA	rs3177928	32,520,413	A	0.047397	0.18	0.002 (0.014)	0.884	0.18	0.074 (0.039)	0.0602	0.172
7	DNAH11	rs12670798	21,573,877	C	0.032634	0.1	-0.01 (0.019)	0.581	0.1	0.016 (0.051)	0.749	0.179
7	NPC1L1	rs217386	44,567,220	G	0.030303	0.48	0.007 (0.026)	0.786	0.48	0.032 (0.069)	0.643	0.187
8	PPP1R3B	rs2126259	9,222,556	C	0.057498	0.99	0.004 (0.062)	0.944	1	-0.543 (0.294)	0.0649	0.189
17	OSBPL7	rs7225700	42,746,803	C	0.022533	0.61	0.003 (0.011)	0.809	0.6	-0.001 (0.03)	0.962	0.194
14	NYNRIN	rs2332328	23,952,898	T	0.030303	0.5	-0.02 (0.114)	0.859	0.5	0.205 (0.254)	0.420	0.199
20	MAFB	rs2902941	38,524,928	A	0.025382	0.72	-0.003 (0.013)	0.807	0.73	0.03 (0.035)	0.381	0.199

Supplementary Table 3: The TG SNPs and their association in Lifelines and PREVEND

TG SNPs						LifeLines			PREVEND			Meta
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE)	P value	RAF	beta (SE)	P value	P value
2	GCKR	rs1260326	27,584,444	T	8.76	0.35	0.027 (0.003)	4.21×10^{-20}	0.37	0.024 (0.006)	1.49×10^{-4}	3.20×10^{-22}
8	LPL	rs12678919	19,888,502	A	13.6	0.9	0.038 (0.005)	9.90×10^{-17}	0.9	0.059 (0.01)	7.70×10^{-9}	1.90×10^{-20}
1	ANGPTL3	rs2131925	62,798,530	T	4.94	0.65	0.023 (0.003)	2.84×10^{-15}	0.65	0.014 (0.007)	0.0307	5.97×10^{-16}
19	APOE–C1–C2	rs439401	50,106,291	C	5.5	0.66	0.021 (0.003)	3.51×10^{-12}	0.66	0.029 (0.006)	5.30×10^{-6}	1.48×10^{-14}
8	TRIB1	rs2954029	126,560,154	A	5.64	0.55	0.017 (0.003)	4.02×10^{-9}	0.56	0.017 (0.006)	5.56×10^{-3}	3.63×10^{-10}
7	MLXIPL	rs7811265	72,572,446	A	7.91	0.87	0.024 (0.004)	7.01×10^{-9}	0.88	0.028 (0.009)	2.13×10^{-3}	4.27×10^{-10}
2	APOB	rs1042034	21,078,786	T	5.99	0.79	0.019 (0.003)	3.86×10^{-8}	0.79	0.022 (0.008)	3.81×10^{-3}	3.17×10^{-9}
15	LIPC	rs261342	56,518,445	G	2.99	0.22	0.018 (0.003)	1.11×10^{-7}	0.22	0.008 (0.008)	0.264	8.15×10^{-8}
4	KLHL8	rs442177	88,249,285	T	2.25	0.57	0.012 (0.003)	2.65×10^{-5}	0.57	-0.001 (0.006)	0.810	5.48×10^{-5}
3	MSL2L1	rs645040	137,409,312	T	2.22	0.79	0.007 (0.004)	0.0343	0.79	0.019 (0.008)	9.61×10^{-3}	6.33×10^{-3}
20	PLTP	rs4810479	43,978,455	C	3.32	0.22	0.009 (0.003)	0.0106	0.21	0.005 (0.008)	0.568	6.47×10^{-3}
2	COBLL1	rs10195252	165,221,337	T	2.01	0.56	0.007 (0.003)	0.0173	0.57	0.008 (0.006)	0.203	6.81×10^{-3}
16	CETP	rs7205804	55,562,390	G	2.88	0.57	0.006 (0.003)	0.0532	0.56	0.012 (0.006)	0.0462	0.0136
5	TIMD4	rs1553318	156,411,901	C	2.63	0.64	0.005 (0.003)	0.0739	0.67	0.017 (0.007)	0.0112	0.0144
11	APOA1–C3–A4–A5	rs964184	116,154,127	G	16.9	0.02	0.02 (0.01)	0.0364	0.02	0.008 (0.022)	0.721	0.0211
11	FADS1–2–3	rs174546	61,326,406	T	3.82	0.21	0.005 (0.004)	0.159	0.22	0.025 (0.008)	1.97×10^{-3}	0.0247
22	PLA2G6	rs5756931	36,875,979	T	1.54	0.68	0.006 (0.003)	0.0527	0.67	0.005 (0.007)	0.490	0.0248

to be continued

continued

TG SNPs						LifeLines			PREVEND			Meta
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE)	P value	RAF	beta (SE)	P value	P value
2	IRS1	rs2943645	226,807,424	T	1.89	0.63	0.006 (0.003)	0.0332	0.63	-0.01 (0.006)	0.132	0.0428
8	PINX1	rs11776767	10,721,339	C	2.01	0.34	0.004 (0.003)	0.167	0.34	0.011 (0.007)	0.0903	0.0457
5	MAP3K1	rs9686661	55,897,543	T	2.57	0.12	0.007 (0.004)	0.126	0.12	0.003 (0.009)	0.740	0.0586
10	CYP26A1	rs2068888	94,829,632	G	2.28	0.51	0.005 (0.003)	0.0669	0.51	-0.009 (0.006)	0.170	0.0656
16	CTF1	rs11649653	30,825,988	C	2.13	0.62	0.003 (0.003)	0.331	0.61	0.013 (0.006)	0.0437	0.0763
6	HLA	rs2247056	31,373,469	C	2.99	0.61	0.004 (0.003)	0.234	0.61	0.006 (0.007)	0.362	0.0793
10	JMJD1C	rs10761731	64,697,616	A	2.38	0.57	0.004 (0.003)	0.182	0.55	0.001 (0.006)	0.899	0.0824
8	NAT2	rs1495743	18,317,580	G	2.97	0.24	0.005 (0.004)	0.191	0.25	0.001 (0.007)	0.871	0.0843
1	GALNT2	rs1321257	228,371,935	G	2.76	0.38	0.002 (0.004)	0.642	0.41	0.021 (0.006)	1.17×10^{-3}	0.102
19	CILP2	rs10401969	19,268,718	T	7.83	1	0.022 (0.023)	0.325	1	0.012 (0.052)	0.821	0.120
12	LRP1	rs11613352	56,078,847	C	2.7	1	-0.009 (0.021)	0.674	1	-0.059 (0.047)	0.206	0.158
12	ZNF664	rs12310367	123,052,631	A	2.42	0.76	0.002 (0.003)	0.526	0.76	0 (0.007)	0.984	0.164
15	FRMD5	rs2929282	42,033,223	T	5.13	0	-0.013 (0.035)	0.709	0	-0.051 (0.088)	0.560	0.177

Supplementary Table 4: The TC SNPs and their association in LifeLines and PREVEND

TC SNPs						LifeLines			PREVEND			Meta
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE)	P value	RAF	beta (SE)	P value	P value
19	APOE–C1–C2	rs4420638	50,114,786	G	0.176897	0.14	0.199 (0.018)	2.74×10^{-27}	0.14	0.135 (0.043)	1.59×10^{-3}	6.71×10^{-29}
19	LDLR	rs6511720	11,063,306	G	0.183631	0.92	0.178 (0.023)	8.25×10^{-15}	0.92	0.202 (0.056)	3.09×10^{-4}	1.32×10^{-16}
11	FADS1–2–3	rs174550	61,328,054	T	0.046102	0.67	0.103 (0.013)	1.52×10^{-15}	0.67	-0.022 (0.031)	0.483	4.39×10^{-14}
1	ANGPTL3	rs3850634	62,823,186	T	0.06734	0.65	0.084 (0.013)	3.58×10^{-11}	0.65	0.065 (0.031)	0.0334	7.76×10^{-12}
5	HMGCR	rs12916	74,692,295	C	0.073556	0.39	0.069 (0.012)	3.78×10^{-8}	0.41	0.077 (0.03)	0.0106	4.79×10^{-8}
2	GCKR	rs1260326	27,584,444	T	0.049469	0.35	0.06 (0.013)	1.82×10^{-6}	0.37	0.134 (0.03)	6.44×10^{-6}	2.53×10^{-8}
2	APOB	rs1367117	21,117,405	A	0.107744	0.32	0.068 (0.013)	1.53×10^{-7}	0.32	0.06 (0.031)	0.0524	4.23×10^{-8}
1	LDLRAP1	rs12027135	25,648,320	T	0.031598	0.56	0.067 (0.012)	3.17×10^{-8}	0.56	0.019 (0.029)	0.51	4.24×10^{-8}
1	SORT1	rs629301	109,619,829	T	0.140119	0.71	0.083 (0.017)	8.20×10^{-7}	0.72	0.113 (0.04)	4.25×10^{-3}	7.78×10^{-8}
8	TRIB1	rs2954022	126,551,803	C	0.05957	0.52	0.048 (0.012)	8.23×10^{-5}	0.53	0.037 (0.029)	0.198	4.06×10^{-5}
8	CYP7A1	rs1030431	59,474,251	A	0.032634	0.33	0.046 (0.013)	3.35×10^{-4}	0.35	0.04 (0.03)	0.184	1.52×10^{-4}
1	IRF2BP2	rs514230	232,925,220	T	0.035224	0.54	0.036 (0.012)	3.01×10^{-3}	0.53	0.088 (0.029)	2.21×10^{-3}	3.55×10^{-4}
20	TOP1	rs4297946	39,244,689	C	0.039368	0.42	0.04 (0.012)	1.29×10^{-3}	0.43	0.032 (0.03)	0.287	6.79×10^{-4}
1	PCSK9	rs2479409	55,277,238	G	0.050764	0.26	0.042 (0.014)	2.89×10^{-3}	0.25	0.052 (0.034)	0.13	1.06×10^{-3}
8	PLEC1	rs11136341	145,115,531	G	0.034706	0.34	0.043 (0.013)	6.95×10^{-4}	0.35	-0.018 (0.031)	0.559	1.25×10^{-3}
5	TIMD4	rs6882076	156,322,875	C	0.051282	0.65	0.034 (0.013)	8.38×10^{-3}	0.75	0.088 (0.034)	9.14×10-3	1.45×10^{-3}
17	OSBPL7	rs7206971	42,780,114	A	0.026159	0.46	0.033 (0.012)	6.08×10^{-3}	0.46	0.036 (0.029)	0.215	2.59×10^{-3}

to be continued

continued

TC SNPs						LifeLines			PREVEND			Meta
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE)	P value	RAF	beta (SE)	P value	P value
6	C6orf106	rs2814982	34,654,538	C	0.048174	0.93	0.076 (0.024)	1.71×10^{-3}	0.95	-0.042 (0.068)	0.534	2.75×10^{-3}
15	LIPC	rs1532085	56,470,658	A	0.039886	0.3	0.047 (0.015)	2.33×10^{-3}	0.31	-0.013 (0.037)	0.72	3.02×10^{-3}
1	MOSC1	rs2807834	219,037,216	G	0.035742	0.78	0.046 (0.017)	7.28×10^{-3}	0.75	-0.02 (0.041)	0.631	8.41×10^{-3}
10	GPAM	rs2255141	113,923,876	A	0.029526	0.27	0.031 (0.014)	0.025	0.28	0.046 (0.033)	0.162	8.97×10^{-3}
8	TRPS1	rs2737229	116,717,740	A	0.028749	0.65	0.031 (0.013)	0.0135	0.66	0.001 (0.031)	0.978	0.0107
6	LPA	rs1564348	160,498,850	C	0.056462	0.07	0.048 (0.024)	0.0464	0.07	0.112 (0.058)	0.0547	0.0123
2	ABCG5/8	rs4299376	43,926,080	G	0.077959	0.12	0.053 (0.02)	7.77×10^{-3}	0.13	-0.08 (0.046)	0.0826	0.0169
16	HPR	rs2000999	70,665,594	A	0.060606	0.41	0.026 (0.013)	0.0477	0.4	0.036 (0.031)	0.256	0.0186
7	DNAH11	rs2285942	21,549,442	T	0.04403	0.13	0.035 (0.018)	0.0512	0.12	0.032 (0.044)	0.459	0.0237
6	FRK	rs9488822	116,419,586	A	0.030562	0.67	0.026 (0.013)	0.0464	0.67	0.013 (0.031)	0.691	0.0254
11	UBASH3B	rs7941030	122,027,585	C	0.025123	0.37	0.027 (0.013)	0.0293	0.36	-0.029 (0.03)	0.333	0.0317
9	ABCA1	rs1883025	106,704,122	C	0.058016	0.75	0.027 (0.016)	0.0861	0.74	0.029 (0.037)	0.431	0.0363
2	RAB3GAP1	rs6759321	136,039,146	T	0.030562	0.16	0.027 (0.017)	0.113	0.18	0.049 (0.038)	0.203	0.0378
12	BRAP	rs11065987	110,556,807	A	0.024864	0.57	0.023 (0.012)	0.0598	0.56	-0.005 (0.03)	0.871	0.0393
18	LIPG	rs7239867	45,418,715	G	0.050246	0.84	0.024 (0.016)	0.14	0.85	0.067 (0.04)	0.0964	0.0393
1	EVI5	rs7515577	92,782,026	A	0.030562	0.93	0.045 (0.025)	0.0655	0.95	0 (0.069)	0.996	0.0397
6	HLA	rs3177928	32,520,413	A	0.059829	0.18	0.019 (0.016)	0.232	0.18	0.104 (0.039)	7.48×10^{-3}	0.0435

to be continued

continued

TC SNPs						LifeLines			PREVEND			Meta
Chr	NearbyGene	SNP	Position	RA	Effect Size	RAF	beta (SE)	P value	RAF	beta (SE)	P value	P value
20	ERGIC3	rs2277862	33,616,196	C	0.030821	0.86	0.029 (0.018)	0.0972	0.86	0.014 (0.042)	0.733	0.0476
7	NPC1L1	rs2072183	44,545,705	C	0.052059	0.19	0.024 (0.016)	0.115	0.19	0.016 (0.037)	0.668	0.0527
3	RAF1	rs2290159	12,603,920	G	0.036778	0.78	0.021 (0.015)	0.158	0.8	0.042 (0.037)	0.253	0.0531
6	HFE	rs1800562	26,201,120	G	0.055944	0.99	0.091 (0.067)	0.172	0.999	0.301 (0.506)	0.553	0.0686
8	NAT2	rs1961456	18,299,989	G	0.027713	0.3	0.02 (0.013)	0.134	0.31	-0.001 (0.031)	0.987	0.0687
16	CETP	rs3764261	55,550,825	A	0.043253	0.32	0.019 (0.013)	0.135	0.33	-0.001 (0.03)	0.964	0.0699
6	MYLIP	rs3757354	16,235,386	C	0.037814	0.78	0.021 (0.015)	0.162	0.78	0.004 (0.036)	0.916	0.0761
11	SPTY2D1	rs10832963	18,620,817	G	0.027454	0.74	0.021 (0.014)	0.124	0.72	-0.025 (0.032)	0.437	0.082
19	FLJ36070	rs492602	53,898,229	G	0.032893	0.52	-0.052 (0.044)	0.24	0.52	0.034 (0.111)	0.760	0.111
9	TTC39B	rs581080	15,295,378	C	0.040663	0.84	0.02 (0.016)	0.234	0.85	-0.019 (0.04)	0.643	0.113
12	HNF1A	rs1169288	119,901,033	C	0.037555	0.33	0.013 (0.014)	0.356	0.31	0.021 (0.034)	0.543	0.117
11	APOA1–C3–A4–A5	rs964184	116,154,127	G	0.121212	0.02	0.043 (0.042)	0.297	0.02	-0.07 (0.102)	0.491	0.137
11	ST3GAL4	rs11220463	125,753,421	T	0.052059	0.04	0.025 (0.031)	0.425	0.04	0.018 (0.071)	0.804	0.141
20	MAFB	rs2902940	38,524,901	A	0.035742	0.73	-0.01 (0.014)	0.467	0.73	-0.001 (0.034)	0.979	0.154
9	ABO	rs651007	135,143,696	T	0.05957	0.06	-0.016 (0.026)	0.546	0.06	-0.033 (0.066)	0.615	0.156
19	PPP1R3B	rs10401969	19,268,718	T	0.122766	0.999	0.002 (0.097)	0.984	0.999	-0.126 (0.246)	0.609	0.199
8	CILP2	rs2126259	9,222,556	C	0.081326	0.99	0.026 (0.068)	0.702	0.99	-0.562 (0.293)	0.055	0.199

Supplementary Table 5: Correlation between lipid risk scores and observed lipid levels in Lifelines and PREVEND

Trait	Weighted Risk Score	Unweighted risk score
LifeLines		
HDL risk (47 snps) vs HDL	r = 0.24 p= 1.5×10^{-139}	r = 0.17 p= 3.2×10^{-70}
LDL risk (37 SNPs) vs LDL	r = 0.19 p= 2.0×10^{-87}	r = 0.15 p = 3.1×10^{-59}
TG risk (30 SNPs) vs TG	r = 0.19 p = 4.2×10^{-91}	r = 0.16 p = 1.2×10^{-60}
TC risk (51 SNPs) vs TC	r = 0.21 p = 1.9×10^{-107}	r = 0.18 p = 6.8×10^{-82}
PREVEND		
HDL risk vs HDL	r = 0.192 p = 3.3×10^{-21}	r = 0.142 p = 2.60×10^{-12}
LDL risk vs LDL	r = 0.138 p = 1.1×10^{-11}	r = 0.111 p = 5.54×10^{-08}
TG risk vs TG	r = 0.209 p = 4.5×10^{-25}	r = 0.166 p = 3.33×10^{-16}
TC risk vs TC	r = 0.144 p = 8.4×10^{-13}	r = 0.107 p = 1.33×10^{-07}

Supplementary Table 6: The Spearman correlation between the weighted risk score and glucose-related traits adjust for covariants

LifeLines		
	FPG Level	HbA1c Level
HDL risk score	r = -0.002 p = 0.81	r = 0.004 p = 0.66
LDL risk score	r = -0.012 p = 0.19	r = -0.009 p = 0.33
TG risk score	r = -0.015 p = 0.13	r = -0.025 p = 0.01
TC risk score	r = -0.007 p =0.47	r = -0.013 p = 0.16
PREVEND		
	FPG (mmol/l)	HOMA
HDL risk score	r = 0.024 p = 0.23	r = 0.037 p = 0.069
LDL risk score	r = 0.046 p = 0.024	r = 0.018 p = 0.39
TG risk score	r = -0.008 p = 0.71	r = 0.035 p = 0.087
TC risk score	r = 0.034 p = 0.095	r = 0.021 p = 0.29

Supplementary Table 7: Single SNP association on glucose-related traits adjust for covariants and lipids

Chr	NearbyGene	SNP	A1	A2	FPG	HBA1C	HOMA	HDL	LDL	TG	TC
2	APOB	rs1042034	T	C	Z = -3.41	Z = -3.78	Z = -2.03	Z = -4.85	Z = 3.42	Z = 5.99	Z = 2.3
					$p = 6.0\times10^{-4}$	$p = 1.6\times10^{-4}$	p = 0.043	$p = 1.50\times10^{-6}$	$p = 5.8\times10^{-4}$	$p = 3.20\times10^{-9}$	p = 0.014
2	GCKR	rs1260326	C	T	Z = 5.9	Z = 0.73	Z = 2.46	Z = 1.19	Z = -4.61	Z = -9.79	Z = -5.64
					$p = 5.40\times10^{-9}$	p = 0.47	p = 0.014	p = 0.098	$p = 4.90\times10^{-6}$	$p = 3.20\times10^{-22}$	$p = 2.50\times10^{-8}$
5	TIMD4	rs1553318	C	G	Z = -0.12	Z = 1.79	Z = -3.21	Z = -1.01	Z = 3.01	Z = 2.29	Z = 2.62
					p = 0.2	p = 0.073	$p = 1.3\times10^{-3}$	p =0.12	$p = 2.2\times10^{-3}$	p = 0.014	$p = 6.4\times10^{-3}$
6	LPA	rs1564348	T	C	Z = 0.36	Z = 0.14	Z = -2.7	Z = 1.03	Z = -2.42	Z = -2.16	Z = -2.36
					p = 0.19	p = 0.89	$p = 7.0\times10^{-3}$	p = 0.12	p = 0.011	p = 0.019	p = 0.012
6	HLA	rs2247056	C	T	Z = -0.47	Z = 0.03	Z = 2.64	Z = 1.11	Z = 1.89	Z = 1.36	Z = 2.09
					p = 0.18	p = 0.98	$p = 8.2\times10^{-3}$	p = 0.11	p = 0.033	p = 0.079	p = 0.022
7	MLXIPL	rs17145738	C	T	Z = -2.8	Z = -2.76	Z = -2.61	Z = -1.55	Z = 0.79	Z = 6.36	Z = 0.98
					$p = 3.9\times10^{-3}$	$p = 5.7\times10^{-3}$	$p = 9.0\times10^{-3}$	p = 0.06	p = 0.15	$p = 3.30\times10^{-10}$	p = 0.12
7	NPC1L1	rs217386	A	G	Z = -0.26	Z = 0.42	Z = -2.71	Z = -0.7	Z = -0.36	Z = -0.56	Z = -0.65
					p = 0.19	p = 0.67	$p = 6.6\times10^{-3}$	p = 0.16	p = 0.19	p = 0.17	p = 0.16
7	MLXIPL	rs7811265	A	G	Z = -2.96	Z = -2.89	Z = -2.72	Z = -1.55	Z = 0.61	Z = 6.32	Z = 0.92
					$p = 2.5\times10^{-3}$	$p = 3.8\times10^{-3}$	$p = 6.5\times10^{-3}$	p = 0.06	p = 0.17	$p = 4.30\times10^{-10}$	p = 0.13
8	CYP7A1	rs1030431	G	A	Z = 2.67	Z = 0.48	Z = 0.41	Z = -0.32	Z = -3.56	Z = -1.93	Z = -3.79
					$p = 5.7\times10^{-3}$	p = 0.63	p = 0.68	p = 0.19	$p = 3.6\times10^{-4}$	p = 0.031	$p = 1.5\times10^{-4}$

to be continued

continued

Chr	NearbyGene	SNP	A1	A2	FPG	HBA1C	HOMA	HDL	LDL	TG	TC
11	FADS1-2-3	rs174550	C	T	Z = -2.52	Z = -2.55	Z = -0.09	Z = -4.57	Z = -7.22	Z = 2.8	Z = -7.63
					$p = 8.4\times10^{-3}$	p = 0.011	p = 0.93	$p = 5.90\times10^{-6}$	$p = 9.80\times10^{-13}$	$p = 4.0\times10^{-3}$	$p = 4.40\times10^{-14}$
12	LRP1	rs3741414	C	T	Z = 1.54	Z = 2.64	Z = -1.4	Z = -2.34	Z = 0.59	Z = 0	Z = -0.01
					p = 0.061	$p = 8.3\times10^{-3}$	p = 0.16	p = 0.013	p = 0.17	p = 0.2	p = 0.2
15	LACTB	rs2652834	G	A	Z = 0.09	Z = -0.7	Z = 3.12	Z = 0.08	Z = 0.19	Z = -0.36	Z = -0.01
					p = 0.2	p = 0.48	$p = 1.8\times10^{-3}$	p = 0.2	p = 0.2	p = 0.19	p = 0.2
16	CETP	rs3764261	A	C	Z = 3.69	Z = 1.38	Z = 5.21	Z = 19.84	Z = -5.87	Z = -3.19	Z = 1.45
					$p = 2.2\times10^{-4}$	p = 0.17	$p = 1.80\times10^{-7}$	$p = 6.10\times10^{-87}$	$p = 6.70\times10^{-9}$	$p = 1.2\times10^{-3}$	p = 0.07
16	CETP	rs7205804	A	G	Z = 3.28	Z = 2.6	Z = 4.16	Z = 17.76	Z = -4.7	Z= -2.32	Z= 1.76
					$p = 9.2\times10^{-4}$	$p = 9.2\times10^{-3}$	$p = 3.20\times10^{-5}$	$p = 6.20\times10^{-70}$	$p = 3.10\times10^{-6}$	p = 0.014	p = 0.042
19	APOE-C1-C2	rs4420638	A	G	Z = 2.92	Z = 3.01	Z = 2.27	Z = 4.5	Z = -13.7	Z= -3.51	Z= -11.25
					$p = 2.8\times10^{-3}$	$p = 2.6\times10^{-3}$	p = 0.023	$p = 8.00\times10^{-6}$	$p = 3.60\times10^{-42}$	$p = 4.2\times10^{-4}$	$p = 6.70\times10^{-29}$
20	TOP1	rs4297946	G	C	Z = -2.57	Z = -0.51	Z = -1.65	Z = 0.17	Z = -2.99	Z = -1.55	Z = -3.37
					$p = 7.4\times10^{-3}$	p = 0.61	p = 0.098	p = 0.2	$p = 2.3\times10^{-3}$	p = 0.06	$p = 6.8\times10^{-4}$
20	PLTP	rs4810479	C	T	Z = -2.95	Z = -3.91	Z = -0.12	Z = -4.44	Z = 1.59	Z = 2.62	Z = 0.35
					$p = 2.5\times10^{-3}$	$p = 9.20\times10^{-5}$	p = 0.9	$p = 1.10\times10^{-5}$	p = 0.057	$p = 6.5\times10^{-3}$	p = 0.19
20	PLTP	rs6065906	C	T	Z = -2.03	Z = -2.94	Z = 0.42	Z = -4.9	Z = 1.47	Z = 3.21	Z = 0.26
					p = 0.026	$p = 3.3\times10^{-3}$	p = 0.67	$p = 1.20\times10^{-6}$	p = 0.068	$p = 1.2\times10^{-3}$	p = 0.19

Supplementary Table 8: Compare the association before or after adjusting for lipids

SNP	A1	A2	After adjusting lipids		Before adjusting lipids	
			Z score	P value	Z score	P value
FPG						
rs1260326	C	T	5.9	5.4×10^{-9}	4.05	5.40×10^{-5}
rs3764261	A	C	3.69	2.2×10^{-4}	1.2	0.097
rs1042034	T	C	-3.41	6.0×10^{-4}	-1.87	0.034
rs7205804	A	G	3.28	9.2×10^{-4}	1.17	0.10
rs7811265	A	G	-2.96	2.5×10^{-3}	-1.81	0.039
rs4810479	C	T	-2.95	2.5×10^{-3}	-2.08	0.023
rs4420638	A	G	2.92	2.8×10^{-3}	1.53	0.062
rs17145738	C	T	-2.8	3.9×10^{-3}	-1.65	0.051
rs1030431	G	A	2.67	5.7×10^{-3}	2.21	0.017
rs4297946	G	C	-2.57	7.4×10^{-3}	-2.81	3.8×10^{-3}
rs174550	C	T	-2.52	8.4×10^{-3}	-1.88	0.034
HbA1c						
rs1042034	T	C	-3.78	1.62×10^{-4}	-2.98	2.9×10^{-3}
rs17145738	C	T	-2.76	5.72×10^{-3}	-2.39	0.017
rs3741414	C	T	2.64	8.3×10^{-3}	2.75	5.9×10^{-3}
rs4420638	A	G	3.01	2.6×10^{-3}	1.25	0.21
rs4810479	C	T	-3.91	9.2×10^{-5}	-3.41	6.6×10^{-4}
rs6065906	C	T	-2.94	3.3×10^{-3}	-2.42	0.016
rs7205804	A	G	2.6	9.2×10^{-3}	1.12	0.26
rs7811265	A	G	-2.89	3.8×10^{-3}	-2.52	0.012
HOMA-IR						
rs1553318	C	G	-3.21	1.3×10^{-3}	-2.1	0.036
rs1564348	T	C	-2.7	7.0×10^{-3}	-3.05	2.3×10^{-3}
rs17145738	C	T	-2.61	9.0×10^{-3}	-1.07	0.28
rs217386	A	G	-2.71	6.6×10^{-3}	-3.03	2.4×10^{-3}
rs2247056	C	T	2.64	8.2×10^{-3}	2.5	0.012
rs2652834	G	A	3.12	1.8×10^{-3}	1.92	0.054
rs3764261	A	C	5.21	1.8×10^{-3}	3.13	1.8×10^{-3}
rs7205804	A	G	4.16	3.2×10^{-3}	1.96	0.05
rs7811265	A	G	-2.72	6.5×10^{-3}	-1.2	0.23

CHAPTER 7

General discussion

Numerous achievements in diabetes have been made for nearly a hundred years, including those that won seven Nobel Prizes. Similar to the work of most medical scientists, our research in this thesis will also contributes to the treatment and prevention of T2D. However, the road to conquer diabetes fully is still very long. Almost every clinical endocrinologist sometimes feels frustrated by diabetes, since we cannot prevent the progress of its chronic complications effectively with our current regimens, in particular the macrovascular complications including cardiovascular diseases. New targets for therapy or prevention still need to be identified to improve the prognosis for T2D.

Another awkward fact about T2D is based on its heterogeneity. Experienced endocrinologists are always frustrated by some well-controlled diabetic patients who progress rapidly to suffer from chronic complications; while a few patients whose plasma glucose levels are continuously high enjoy their lives freely without complications. Hence, this hetereogeneity only seems to be explained by genetics. However, we cannot differentiate between these two types of diabetic patients when they are diagnosed with T2D. What we can do is use a trial-and-error method, adjusting each regimen according to each patient's response in order to make the patient's regimen as near perfect as possible, which is called "personalized medicine". Eventually, endocrinologists hope to have a method and especially some biomarkers to predict these situations, which new developments in genetics might provide some chance of finding, fortunately.

1. Personalized Medicine in Ancient Times

Personalized medicine, according to its literal meaning, is patient-centric medicine. Hence, personalized medicine dates back to ancient Greek times, when medicine was separated from witchcraft. "The physician must not only be prepared to do what is right himself, but also to make the patient, the attendants, and externals cooperate", Hippocrates said[1]. Therefore, personalized medicine in fact appeared when medicine was introduced.

In ancient China, "treat the same disease with different regimens, while treating different diseases with the same regimen" was one of the most important principles of Chinese traditional medicine. The essence of this principle is that the correct treatment is based on careful observation of the patient and analysis, not

merely experience. For instance, a patient considered to be diagnosed with diabetes would often be classified as “Up Jiao”, “Middle Jiao” and “Down Jiao” after carefully taking a case history and making a physical examination. If a patient was diagnosed as “Down Jiao”, he was believed to suffer from diabetic nephropathy and his regimen would be different from that prescribed for patients of the other two categories.

At that time, the main problems of personalized medicine were lack of information for an accurate diagnosis and a lack of effective treatments. Some regimens of Chinese traditional medicine could alleviate the symptoms of diabetes but could not effectively lower blood glucose, so that those treatments could not delay the progress of diabetes.

Thus, we have had personalized medicine since ancient times, but few effective methods for diagnosis and few effective treatments.

2. Personalized medicine in modern medical practice

There has been much progress made in medicine in the past 100 years and the progress in basic science has provided us with not only the fields of physiology and pathophysiology, but also various types of methods of diagnosis and treatments. This progress has made personalized medicine more important and effective.

2.1 Accurate classifications of diseases are one big step of personalized medicine

Correct classifications of diseases are very important for personalized medicine. In Ancient time, diseases always were diagnosed only according to symptoms and clinical signs. Hence quite a few of patients would be misdiagnosed and personalized medicine could not be effective. In ancient China, thirsty and emaciation were characteristics of diabetes; but sometimes patients of untypical hyperthyroidism would be misdiagnosed as diabetes so that they could not be correctly treated by personalized medicine. However, measurement of blood glucose levels gave us an accurate method of differential diagnosis so that we can effectively treat diabetic patients. Classification of T1D and T2D by Himsworth in 1936 was also very important for personalized medicine, so that oral anti-diabetic drugs could be used only for patients of T2D correctly.

2.2 Heterogeneity of complex diseases and importance of personalized medicine

Personalized medicine is especially important for complex diseases like T2D. T2D is a typically heterogeneous disease, which is actually a group of metabolic disorders with the same characteristics of raised blood glucose levels. We have to use the nomination of "T2D" because we do not know enough to make a more detailed and accurate classification. The current classification of diabetes for clinical endocrinologists includes T1D, T2D, gestational diabetes, and other specific types of diabetes. When the mechanism of one group of special diabetic patients was discovered, such as maturity-onset diabetes of the young (MODY), this subgroup was then excluded from T2D and reclassified as a specific subtype of diabetes. Although progress in genetics and other subjects have identified some specific subtypes of diabetes, the remaining group of T2D patients is still heterogeneous. For some patients, beta cell dysfunction is the main cause of T2D, while for other patients, IR may be the most important cause. Different situations need different treatments. As described in Chapter 1, there are several options for treating T2D; hence we always have to use the old method of "trial-and-error" (observation and adjustment) to attain the goal of personalized medicine.

3. Current Situations for Personalized Medicine

3.1 Flourishing of evidence-based medicine (EBM) and rise of personalized medicine

EBM has been the main stream in modern medical practice since the 1990s and had a positive influence on people's health[2]. Almost every common disease has its own guidelines for diagnosis and treatment; these effectively steer professional medical behavior worldwide. However, sometimes the guidelines are mis-used and principles of personalized medicine are neglected. In Italy, a similar percentage of various regimens for T2D were adopted in patient populations of different ages and even with different renal function[3]. This will lead to poorer outcomes. Such situations also exist in other countries[4]. American Diabetes Association and European Association for the Study of Diabetes therefore gave their annual position statement a new name of "a patient-centered approach"[5]. Furthermore, we should be very careful to evaluate a new drug in clinical trials on specific patient cohorts.

For instance, an example from another disease is Trastuzumab, a monoclonal antibody that binds the *HER2*/neu receptor: it is effective for the patients of breast cancer with overexpression of *HER2*. However, such patients are generally less than 30% of all patients with breast cancer[6]. If we only use data from the clinical trials for general populations of breast cancer patients, this effect would never have been discovered. Therefore, personalized medicine is more and more important for clinical behavior and research.

3.2 Basic medicine offers possibility of detailed classifications and new treatments

As described in 2.2 of this chapter, for personalized medicine it is important to classify diseases into detailed categories, so that treatments will be appropriate. Progress in basic medicine continues to help us make better and better classifications according to the disease etiology. In Chapter 5, we summarized a group of patients with insulin-related IgG-induced dysglycemia and reclassified them according to types of antibody, antigen, and antigen epitopes. Although this classification cannot directly guide the treatment of these diseases today, in the near future, when we can determine each antigen epitope of these patients and when we have various types of insulin analogues, it should be effective. In Chapter 4, we described some patients with insulin allergy. Though we succeeded in using desensitization by CSII, the best treatment for these cases is to determine which specific epitope of insulin induces allergy for each patient, and use the personalized insulin analogue without that specific epitope.

Sometimes basic medicine is especially effective for the personalized treatment of some rare diseases. In Chapter 4, we described two rare cases. We used column chromatography to help identify the existence of insulin-binding proteins in the first case, and we applied the recovery test in the second case to implicate the existence of the insulin-antibody complex, although the serum insulin level was zero. Although both cases were effectively treated by immunosuppressive therapy, the personalized design for each case was the key to making an accurate diagnosis.

3.3 Genetics and personalized medicine

Rapid progress in genetics has revealed the mechanisms of many diseases, especially monogenic diseases. For diabetes, at least 27 monogenic subtypes have been identified so far[7]. As mentioned before, this is a big step towards a more

personalized treatment.

The most successful example is neonatal diabetes mellitus (NDM). Although NDM was recognized more than 200 years ago, genetic etiologies for NDM have mainly been defined in over the past 20 years[8]. Several genes were identified for NDM, including *IPF1, GCK, PTF1A*, and *GLIS3*. In 2004, *KCNJ11* was identified as one of causal genes of NDM in a case report[9]. Though scientists speculated that NDM was different from type 1 diabetes, the regimen of insulin therapy is generally accepted as the most appropriate treatment. However, the cases of mutations in *KCNJ11* or *ABCC8* can be successfully treated by sulfonylurea instead of insulin. *KCNJ11* encodes one subunit of the ATP-sensitive potassium (K_{ATP}) channel, while *ABCC8* encodes other subunit, which is also the receptor for sulfonylurea drugs. Hence it is possible to treat these two types of NDM with sulfonylurea drugs, and subsequent clinical reports confirmed the effect[10]. Every patient with NDM should be tested for these two types of mutations before proposing a therapy regimen. Cost-effectiveness studies also showed that genetic testing could lower the total cost of treatment[11]. Therefore, *KCNJ11* and *ABCC8* are now tested as new biomarkers for this specific subtype of NDM, which illustrates a successful example of personalized medicine.

MODY is another example of personalized medicine. Although MODY was seen as a specific subtype of T2D for a long time, because age of onset was in adolescence, the principle of treatment for MODY was not very different to that for ordinary T2D patients. However, when the causal genes for various subtypes of MODY were identified, personalized treatments were applied and their effects improved greatly. MODY2 is caused by mutations of glucokinase, a key enzyme of glycolysis – a slightly higher blood glucose levels will accelerate glycolysis. MODY1 and MODY3 are due to mutations of *HNF4alpha* and *HNF1alpha*, respectively: both mutations can increase sensitivity to sulfonylurea drugs. Hence, the treatment for MODY2 is not pharmacotherapy, except if the patient is pregnant, but simple lifestyle intervention. The first choice of treatments for MODY1 and MODY3 should be low-dosage sulfonylurea. MODY1, MODY2, and MODY3 account for 87% of all MODY patients and this personalized therapy means that most MODY patients are given the most appropriate treatment[12].

Apart from unraveling monogenic diabetes, genetic studies have also made

progress in other respects. The most influential progress was the completion of the Human Genome Project (HGP), which made personalized medicine a hot topic.

4. Personalized medicine reinterpretated by NIH: waiting to be decoded

Personalized medicine has become increasingly popular in the past 15 years because of the Human Genome Project, which was completed in 2000. According to bibliometric studies, the amount of literature on "personalized medicine" increased rapidly just after the completion of HGP[13]. The US National Institutes of Health (NIH) defined personalized medicine as "an emerging practice of medicine that uses an individual's genetic profile to guide decisions made in regard to the prevention, diagnosis, and treatment of disease"[14].

Compared to past eras, the current problem of personalized medicine is not due to limited methods, but to too much information that we cannot (yet) understand. It is similar to having an encyclopedia written in ancient Egyptian hieroglyphics and waiting for it to be decoded. More and more studies are now focusing on how to understand and exploit all the genetic and genomic information available.

5. Personalized Medicine in the Near Future: Pharmacogenetics and Pharmacogenomics

As an important branch of genetics, pharmacogenetics aims to study how genetic variation affects drug response, including pharmacokinetics, pharmacodynamics, and side-effects[15]. Before the HGP, pharmacogenetics had revealed a lot of information about personalized medicine, while pharmacogenomics was using genomic technology to extend pharmacogenetics since the completion of the HGP[16]. For diabetes, there have been quite a few pharmacogenetic and pharmacogenomic studies on oral anti-diabetic drugs[17,18] and some examples of pharmacogenetic studies are given below.

5.1 Sulfonylureas

KCNJ11 and *ABCC8* encode two subunits of the K_{ATP} channel of beta cells. Hence, it is easy to understand that polymorphisms of these two genes will alter the effects of sulfonylurea drugs[10,19]. However, *CYP2C9* is a gene encoding a

rate-limiting enzyme that can eliminate sulfonylureas, which could affect their hypoglycemic effects by altering their blood concentration[20,21]. If, in an individual patient, one type of sulfonylurea is both slowly eliminated and sensitive to the K_{ATP} channel, sulfonylurea drugs will be quite an effective treatment for him/her, but it will also be easy to induce severe hypoglycemia. If we have enough information about these genes, we can avoid many hypoglycemic attacks occurring as an adverse reaction to sulfonylurea treatment.

5.2 Glinides

Glinides also stimulate insulin secretion by acting on the K_{ATP} channel of beta cells. For repaglinide, polymorphisms of *CYP2C8* and *SLCO1B1* affect the plasma concentration[22,23], while polymorphisms of KCNJ11 affect the therapeutic response[24]. There are relatively few pharmacogenetic studies on nateglinide compared to repaglinide, and only *CYP2C9* and SLCO1B1 polymorphisms may affect the therapeutic response[25,26].

5.3 Metformin

In data from the Diabetes Prevention Program (DPP), *MATE1* gene polymorphisms were found to be associated with insensitivity to metformin[27]. GWAS showed that rs112112617 in the *ATM* locus was related to metformin treatment success[28], while *OCT1* is a gene that can influence the plasma concentration of metformin[29-31]. Since we do not know enough about how metformin works, these two genes (particularly *ATM*) will be used not only in personalized medicine, but also to investigate further mechanisms of diabetes etiology.

5.4 Thiazolidinediones

Pharmacodynamic pharmacogenetic studies showed that genes encoding PPARgamma, lipoprotein lipase, adiponectin, and resistin were associated with the effect of pioglitazone therapy[32,33], while a *CYP2C8* polymorphism is associated with the plasma concentration of pioglitzaone[34].

Pharmacogenetic and pharmacogenomic data can provide more and more pharmacologic data on an individual (such as pharmacodynamics, pharmacokinetics, side-effects, etc.), which will make personalized pharmacotherapy more effective and decrease the adverse side-effects of drugs dramatically.

6. Using Personalized Medicine in Prevention

In Chapter 2, we identified approximately 50 genes related to T2D. The more the risk alleles one individual carries, the higher the chance he/she will suffer from T2D. However, this does not mean that we can predict the development of T2D only from genotypic data, since environmental factors and gene-environment interactions are also very important for its prediction. For example, Pima Indians, who are believed to have a susceptible genotype for T2D, have a much lower prevalence of T2D in northern Mexico than in the nearby southwest USA[35]. Hence, it is inappropriate for personalized medicine to predict the development of T2D only from genes related to the disease, although in population-based studies, the predictive effect will be more accurate when genetic factors are added to the other known predictive factors[36].

In contrast, lifestyle interventions and metformin have proved they can be used to prevent T2D effectively[37]. Since individual responses to metformin are related to genotype, it is better to choose metformin as a course of treatment for those individuals with alleles which can produce good effects from metformin. If an individual does not have a beneficial effect for metformin, he/she should be encouraged to adopt lifestyle interventions. However, we do not yet have enough data to prove whether successful lifestyle changes are related to certain genetic polymorphisms.

7. Personalized Medicine and Other "Omics" Data

After high-throughput techniques were successfully used in genomics, similar techniques were applied to other fields, leading to transcriptomics, proteomics, lipidomics, metabolomics, etc. This means that we can now study the data of RNA, proteins, lipids, metabolites, etc., and we can also acquire epigenetic information to compose "epigenomic profiles" by similar techniques. Genomics provides us with information on someone's DNA, which is relatively constant in an individual, but other "omics" data can give us more instant information at a certain time and state. The major problem today is that we cannot effectively explore all this data. With further progress in bioinformatics, and once we can decode various types of information, personalized medicine will become a much more powerful tool.

8. Systems Biology: Research into Mechanisms and Personalized Medicine

As Margaret A. Hamburg and Francis S. Collins described[38], "we are now building a national highway system for personalized medicine", which means that our current research is similar to creating a network-like system with our "omics" databases for complex diseases, such as T2D. The challenge we are now facing is how to understand various types of data as an integrated system instead of millions of bits of detailed information. This is what systems biology methods can help us achieve.

In Chapter 1, we briefly introduced the ideas and goals of systems biology. Although systems science became a recognized discipline some 60 years ago, systems biology was only adopted by medical research less than 15 years ago, almost at the same time as the start of the HGP.

In spite of many different definitions, systems biology approaches explore the huge complexity of biological systems. Compared to traditional methods, which are like "blind men feeling an elephant", systems biology approaches aim to understand biological systems from the insights provided by systems science. These are similar to seeing the whole elephant as described by blind men. Since "omics" databases have provided us with information from various levels of human biology, the most important topic of systems biology has become how to discover the complex interactions.

Diabetes is a special disease for systems biology: glucose metabolism has its own negative-feedback systems and interacts with other feedback systems, such as lipid metabolism, the renin-angiotensin-aldosterone system, etc. Although we have a deep understanding of many details of the pathophysiological process of diabetes, it is far from enough for providing true personalized medicine. Thus, systems biology should be applied to the interactions of various kinds of information. In Chapter 6, we investigated the pleiotropic effects of lipid genes on glucose-related traits, and surprisingly found their direct, although weak, effect is to prevent blood glucose from increasing. This example reveals the complexity of genes and their actions, and it would be beneficial to investigate the further mechanisms. As more and more mechanisms are discovered, we can attain the "P4" level of medicine

(predictive, preventive, personalized, participatory) that systems biology offers, especially in relation to personalized medicine[39].

9. Conclusion

Although personalized medicine was first practiced in ancient times, it has been endowed with more and more connotations following the progress of modern science and technology. To be able to make a detailed classification of subgroups of patients is a big step towards offering personalized medicine. In contrast to earlier times, in the current post-GWAS era, we are now seeing huge amounts of "omics" data that we cannot yet understand, although we know it will eventually help us to offer personalized medicine. Our major challenge is how to "decode" these data, and this is dependent on the progress being made in systems biology, which aims at understanding the complexity of biological systems from insights gained by systems science.

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Summary

Type 2 diabetes (T2D) has become one of the most common chronic diseases worldwide in the past 20-30 years. Although the discovery of insulin was an epoch-making achievement in the history of treating diabetes, we still face problems that insulin treatment cannot solve. And there are other, new, problems arising as side-effects from the use of insulin, for example insulin-induced hypoglycemia. The crucial issue is that we still cannot prevent the chronic and often severe complications of T2D, nor have we found an ideal way to prevent T2D developing. It is vitally important to investigate the mechanisms of T2D, so that we can fight the disease more effectively in the near future. In this thesis I look at novel mechanisms which may lead to prevention and treatment of T2D, and I describe investigations into some of these mechanisms from different pespectives, including important findings from genetic studies.

In Chapter 1 I give a brief historical overview of the therapeutics of diabetes since insulin was introduced for clinical application. Insulin has significantly extended the lives of diabetes patients, but it has also provided clinical doctors and scientists with the chance to observe the outcome of chronic diabetic complications, which still remain unresolved. We need new drugs to prevent and treat T2D effectively, and therefore we need to investigate more of the mechanisms active in T2D, especially from the genetics viewpoint. Insulin can cause serious side-effects, which, although not as common as when it was first introduced as a treatment, still remain a problem. To solve these problems we need to discover the mechanisms of T2D and understand them better.

In Chapter 2, I report a review of 50 loci associated with T2D; these were mainly discovered by genome-wide association studies. The new candidate genes thus identified will be potential targets for anti-diabetic drugs in the future. Based on our current knowledge of their presumed molecular functions, we have classified them into various categories and discuss the relationships between the functions of each category and the current treatments for T2D.

In Chapter 3, I discuss whether hypertriglyceridemia and low-HDL cholesterol can be causal factors in the development of insulin resistance. If this is true, we

could prevent T2D by intervening in the hyper¬triglyceridemia and low-HDL cholesterol, which would represent good progress towards preventing T2D. I report a review of the evidence from epidemiological, genetic and intervention studies that supports this hypothesis.

Chapters 4 and 5 cover treatment of the side-effects caused by insulin therapy. In Chapter 4, I introduce CSII as a method of desensitization therapy as an alternative to multi-dose injections in T2D patients with insulin allergy. Although CSII has been used to treat insulin allergy for more than 20 years, the patients who could tolerate insulin therapy by CSII have to use it for the rest of their life. While our cases show success in desensitization by CSII, they also indicate a novel mechanism of T2D related to immune tolerance. In Chapter 5 I describe two cases with contrasting clinical symptoms, but due to similar mechanisms, with both related to anti-insulin antibody (AIA)-induced dysglycemia. Case 1 is a patient with severe hypoglycemia but no anti-insulin antibody could be detected in his serum, and he was eventually correctly diagnosed based on the results of column chromatography. Case 2 is a patient with severe hyperglycemia. His daily insulin dosage reached 650-800 IU, but his serum insulin was zero. We used a recovery test to show the existence of insulin in his serum. His treatments were similar to immune-suppressive regimens. Hence, we suggest a new classification for insulin-related antibody-induced dysglycemia that is according to the pathophysiological mechanisms involved.

In Chapter 6, I report an investigation into the effects of lipid genes on glucose-related traits. In contrast to the strong correlation between dyslipidemia and glucose-related traits at a clinical level, the genetic components they share are surprisingly few. Genome-wide association studies have identified 95 loci that explain a substantial proportion of variance in blood lipids, which makes it possible to detect their effects on glucose-related traits. In this study, we focused on 95 lipid loci and tested their association collectively and individually with fasting glucose, glycated hemoglobin (HbA1c), and insulin resistance in two independent cohorts (10,995 subjects from LifeLines and 2,438 subjects from PREVEND). However, in contrast to their phenotypic relationship, the genetic predisposition to dyslipidemia showed a pleiotropic effect in lowering the levels of glucose traits. After adjusting for circulating lipid levels, higher TG, LDL and TC risk scores, and a lower HDL

risk score were correlated with lower levels of fasting plasma glucose, HbA1c and HOMA-IR. Furthermore, at single SNP level, 15 lipid loci showed pleiotropic association with glucose traits at P = 0.01 level and eight of them (*CETP, MLXIPL, PILP, GCKR, APOB, APOE-C1-C2, CYP7A1* and *TIMD4*) had an opposing allelic direction on dyslipidemia and glucose levels. These unexpected results suggest a complex genetic regulation of the lipids and glucose metabolism, and a complex metabolic interplay between them.

In Chapter 7 I introduce a brief history of personalized medicine and discuss the contributions made by the research reported in this thesis to current and future personalized medicine strategies.

In conclusion, the work in this thesis shows our endeavors, in several respects, in investigating mechanisms for preventing and treating T2D. The paradoxical finding that a genetic predisposition to dyslipidemia shows a pleiotropic effect to lowering the levels of glucose traits is expected to lead to novel mechanistic insights into this disease. However, the effect sizes are still modest and they cannot be applied in clinical medicine yet. There is still much to do before the common, chronic disease of type 2 diabetes is defeated.

Samenvatting

Type 2 diabetes (T2D) is wereldwijd een van de meest voorkomende chronische ziektes geworden in de laatste 20-30 jaar. Alhoewel de ontdekking van insuline een gigantisch succes is in de geschiedenis van diabetes behandeling, moeten we nog verschillende problemen overkomen die insuline nog niet heeft opgelost. Daarnaast zijn er een aantal nieuwe problemen opgedoken als bijwerkingen van insuline gebruik zoals bijvoorbeeld insuline geïnduceerde hypoglycemie. Het cruciale probleem is dat we de chronische en vaak ernstige complicaties van T2D niet kunnen voorkomen en we ook ideale methode ter preventie van T2D ontwikkeling nog niet hebben gevonden. Het is van vitaal belang om nieuwe mechanismen van T2D te onderzoeken zodat we T2D effectiever kunnen bestrijden in de nabije toekomst. In dit proefschrift bekijk ik nieuwe mechanismen voor preventie en behandeling van T2D, en beschrijf ik een aantal studies naar nieuwe mechanismen vanuit verschillende perspectieven inclusief belangrijke bevindingen vanuit genetische studies.

In hoofdstuk 1 geef ik een kort historisch overzicht van diabetes therapie sinds de introductie van insuline in de kliniek. Insuline heeft het leven van diabetes patiënten significant verlengd, maar het heeft klinische dokters en wetenschappers de mogelijkheid gegeven om de complicaties die gepaard gaan met chronische diabetes te observeren. Deze complicaties zijn tot op dit moment nog niet opgelost. Om T2D effectief te voorkomen en behandelen is er een dringende behoefte aan nieuwe medicijnen. Hiervoor is meer onderzoek nodig naar mechanismen die actief zijn in T2D, vooral vanuit de genetisch oogpunt. Insuline kan serieuze bijwerkingen veroorzaken, die, alhoewel niet zo frequent als eerder, nog steeds een probleem vormen. Om oplossingen te vinden voor deze problemen moeten we eerst de mechanismen die aan T2D ten grondslag liggen ontdekken en ze beter begrijpen.

In Hoofdstuk 2 geef ik een overzicht van 50 loci geassocieerd met T2D die vooral ontdekt zijn door genoom-wijde associatie studies. De nieuw geïdentificeerde kandidaat genen zijn potentiële doelwitten van anti-diabetische medicijnen in de toekomst. Gebaseerd op onze huidige kennis van hun veronderstelde moleculaire functie, hebben we ze ingedeeld in verschillende categorieën en bespreken we de

relatie tussen functie van elke categorie en de huidige behandeling van T2D.

In hoofdstuk 3 bespreek ik of hypertriglycemie en laag-HDL cholesterol causale factoren kunnen zijn in de ontwikkeling van insuline resistentie. Als dit klopt, dan kunnen we een flinke voortgang boeken in de preventie van T2D door in te grijpen in de hypertriglycemie en laag-HDL cholesterol. Ik geef een overzicht van bewijs van epidemiologische, genetische en interventie studies die deze theorie ondersteunen.

Hoofdstuk 4 en 5 beslaan de behandeling van bijwerkingen veroorzaakt door insuline therapie. In hoofdstuk 4 introduceer ik CSII, een desensitisatie therapie als een alternatief voor de veelvoudige dosis injecties in T2D patiënten met insuline allergie. Alhoewel CSII al voor meer dan 20 jaar wordt gebruikt als behandeling voor insuline allergie, moeten de patiënten die insuline therapie door CSII tolereren het de rest van hun leven blijven gebruiken. Terwijl onze cases succes laten zien in desensitisatie door CSII, is er ook een indicatie van een nieuw mechanisme van T2D gerelateerd aan immuun tolerantie. In hoofdstuk 5 beschrijf ik twee cases met tegenstrijdige klinische symptomen, maar door een vergelijkbaar mechanisme die beide gerelateerd zijn aan anti-insuline antilichamen (AIA)-geïnduceerde dysglycemie. Case 1 is een patiënt met ernstige hypoglycemie maar anti-insuline antilichamen konden niet worden aangetoond in zijn serum. De patiënt werd uiteindelijk correct gediagnosticeerd op basis van kolom chromatografie. Case 2 is een patiënt met ernstige hyperglycemie. Zijn dagelijkse insuline dosis bereikte 650-800 IU, maar zijn serum insuline was nul. We hebben een herstel test gebruikt om de aanwezigheid van insuline in zijn serum aan te tonen. Zijn behandeling was vergelijkbaar met immuun onderdrukkende therapie. Derhalve stellen we een nieuwe classificatie van insuline-gerelateerde antilichaam- geïnduceerde dysglycemie voor op basis van betrokken pathofysiologische mechanismen.

In hoofdstuk 6 doe ik verslag van een onderzoek dat in gaat op de effecten van lipiden op glucose-gerelateerde kenmerken. In tegenstelling tot de sterke correlatie tussen dyslipidemie en glucose-gerelateerde kenmerken op het klinische niveau, zijn de overeenkomsten op genetisch niveau verrassend beperkt. Genoom-wijde associatie studies hebben 95 loci geïdentificeerd die een substantieel deel van de variatie in bloed lipiden verklaren, wat het mogelijk maakt om het effect op glucose-gerelateerde eigenschappen te detecteren. In deze studie hebben we

ons geconcentreerd op 95 lipiden loci en hebben we hun associatie gezamenlijk en individueel met gevast bloedsuikerspiegel, geglycosyleerde hemoglobine (HbA1c), en insuline resistentie in twee onafhankelijke cohorts (10.995 individuen van Lifelines en 2.438 individuen van PREVEND) bepaald. Echter, in tegenstelling tot hun fenotypische relatie, laat de genetische predispositie tot dyslipedemie een pleiotropisch effect zien in verlaging van glucose kenmerken. Na aanpassing van de circulerende lipide spiegels, werden hogere TG, LDL en TC risico scores, en een lagere HDL risico score gecorreleerd aan lagere spiegels van gevast bloedsuiker, HbA1c en HOMA-IR. Bovendien, op enkel SNP niveau, 15 lipide loci lieten een pleiotropische associatie zien met glucose kenmerken op $P = 0.01$ niveau en 8 van deze genen (*CETP, MLXIPL, PILP, GCKR, APOB, APOE-C1-C2, CYP7A1 en TIMD4*) hadden een tegengesteld effect op dyslipidemie en glucose spiegels. Deze onverwachte effecten suggereren een complexe genetische regulatie en metabole interactie van lipiden en glucose metabolisme.

In hoofdstuk 7 introduceer ik kort de geschiedenis van persoonsgebonden geneeskunde en bespreek ik de bijdrage van onderzoek gerapporteerd in dit proefschrift aan huidige en toekomstige strategieën van persoonsgebonden geneeskunde.

In conclusie, het werk in dit proefschrift toont onze pogingen, om vanuit verschillende aspecten, mechanismen te onderzoeken ter preventie en behandeling van T2D. De paradoxale bevinding dat een genetische predispositie tot dyslipidemie een pleiotropisch effect op verlaging van glucose kenmerken laat zien, wekt de verwachting dat dit tot nieuwe mechanistische inzichten kan leiden van deze ziekte. Alhoewel, de effecten nog bescheiden zijn, kunnen ze op dit moment nog niet worden toegepast in de kliniek. Er is nog steeds veel te doen voordat de veel voorkomende chronische ziekte diabetes verslagen is.

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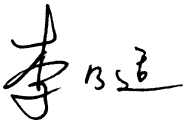

Curriculum Vitae

Naishi Li was born on November 3, 1974 in Yangzhou, China. After graduation from Yangzhou high school of Jiangsu Province, Naishi Li was admitted to Peking Union Medical College (PUMC, in Beijing, China) in 1992. He received premedical training (1992-1995) in college of life science, Peking University (Beijing, China). Then he studied in PUMC, and graduated with the Medical Doctor Degree in 2000. During the last year in PUMC, he completed his thesis on serum IGFBP1 levels of Chinese obese patients under supervision of prof. Yifan Shi and prof. Jieying Deng, professors of department of endocrinology at Peking Union Medical College Hospital (PUMCH). Since then, he had been working as a clinical doctor of endocrinology in PUMCH for more than 11 years and did clinical research on obesity, diabetes mellitus, insulin allergy, short statue and some puberty disorders. He started his PhD project in January 2012 at University Medical Center Groningen, Department of Molecular Genetics. He works under supervision of dr. Jingyuan Fu and prof. dr. Marten H. Hofker, and the results of PhD research are presented in this thesis.

List of Publications

English Journal papers

1. **Li N**[*], van der Sijde MR[*], LifeLines Cohort Study, Bakker SJ, Dullaart RP, van der Harst P, Gansevoort RT, Elbers CC, Wijmenga C, Snieder H, Hofker MH, Fu J (2013) Genetic predisposition to dyslipidemia pleiotropically lowers plasma glucose, HbA1c and HOMA-IR levels. *Submitted*. ([*]These authors contributed equally)

2. **Li N**, Fu J, Koonen DP, Kuivenhoven JA, Snieder H, Hofker MH (2013) Are Hypertriglyceridemia and low HDL Causal Factors in the Development of Insulin Resistance? *Conditionally accepted*

3. Zhu HJ, Ding HH, Deng JY, Pan H, Wang LJ, **Li NS**, Wang XQ, Shi YF, Gong FY (2013) Inhibition of preadipocyte differentiation and adipogenesis by zinc-2-glycoprotein treatment in 3T3-L1 cells. J Diabetes Invest 4 (3):252-260

4. Zhang DM, Xue HD, Duan L, Li J, **Li NS**, Jin ZY (2013) A Small Solitary Pulmonary Nodule Discovered by (18)F-fluorodeoxyglucose Positron Emission Tomography and CT: Rare Infection Instead of Rare Tumor. Chin Med Sci J 27 (4):249-252.

5. Zhu HJ, Dong CX, Pan H, Ping XC, **Li NS**, Dai YF, Wang LJ, Yang HB, Zhao WG, Gong FY (2012) rs4215 SNP in zinc-alpha2-glycoprotein gene is associated with obesity in Chinese north Han population. Gene 500 (2):211-215.

6. Pan H, Ping XC, Zhu HJ, Gong FY, Dong CX, **Li NS**, Wang LJ, Yang HB (2012) Association of myostatin gene polymorphisms with obesity in Chinese north Han human subjects. Gene 494 (2):237-241.

7. Zhu HJ, Dai YF, Wang O, Li M, Lu L, Zhao WG, Xing XP, Pan H, **Li NS**, Gong FY (2011) Generalized glucocorticoid resistance accompanied with an adrenocortical adenoma and caused by a novel point mutation of human glucocorticoid receptor gene. Chin Med J (Engl) 124 (4):551-555

8. **Li N** (2011) Liu Shih-Hao: pioneer of translational medicine in China. Sci China Life Sci 54 (12):1089-1095.

9. Zhu Y, **Li NS**[$], Lu L, Chen S, Xing XP, Meng XW, Guan H, Tan L, Lu K, Meng YX (2010) Hypercalcemic crisis due to a mediastinal parathyroid cyst diagnosed by ultrasound-guided fine needle aspiration. Chin Med J (Engl) 123 (24):3731-3733 ([$]corresponding author)

10. Zhao Y, He X, Huang C, Fu X, Shi X, Wu Y, Han Y, **Li N**, Heng CK (2010) The impacts of thiazolidinediones on circulating C-reactive protein levels in different diseases: a meta-analysis. Diabetes Res Clin Pract 90 (3):279-287.

11. Zhang D, **Li N**[$] (2010) Professor Liu Shih-hao and the first case study of insulinoma in China. Chin Med Sci J 25 (4):246-249. ([$]corresponding author)

12. Gong FY, Zhang SJ, Deng JY, Zhu HJ, Pan H, **Li NS**, Shi YF (2009) Zinc-alpha2-glycoprotein is involved in regulation of body weight through inhibition of lipogenic enzymes in adipose tissue. Int J Obes (Lond) 33 (9):1023-1030.

13. Zhang S, Li YX, **Li NS**, Li WH, Zhu HJ, Gu F, Wang H (2009) Octreotide acetate long-acting release in treatment of pancreatic neuroendocrine tumors. Chin Med J (Engl) 122 (13):1582-1584

14. Yang H, **Li N**[$], Xu L, Xia W (2009) Severe hydronephrosis in nephrogenic diabetes insipidus. Clin Med Res 7 (4):170-171. ([$]corresponding author)

15. Zhang Y, Xiao X, Liu Y, Zhu X, Li W, **Li N**, Yuan T, Wang H (2008) The association of the PAX4 gene with type 1 diabetes in Han Chinese. Diabetes Res Clin Pract 81 (3):365-369.

Book chapters:

1. Wolfs M*, **Li N***, Fu J, Wijmenga C, van Haeften TW, Hofker MH. (2013) Genetic Insights Through Genome Wide Association Studies in Type 2 Diabetes Mellitus will Lead to New Therapeutics. Changing Views on Living Organisms, vol 1, 1 edn. Bentham eBooks, pp 210-240 (*These authors contributed equally)

2. **Li N** (2009) Pituitary diseases. In: Sung JJ, Wang JY, Shen T (ed) Essential Internal Medicine. 1 edn. People's Medical Publishing House, Beijing, pp 432-438

3. **Li N** (2009) Disorders of sex hormone. In: Sung JJ, Wang JY, Shen T (ed) Essential Internal Medicine. 1 edn. People's Medical Publishing House, Beijing, pp 451-456

English Presentations (1st author)

1. **Li N**, Gong F, Li Y, Zhu H, Li M, Zhang D, Pan H. Serum INSL3 concentrations increase during male puberty in the Mongolian population of East Asia. 9th Joint Meeting of Paediatric Endocrinology (ESPE – PES – APEG – APPES – ASPAE – JSPE - SLEP), September 19 - 22, 2013, Milan, Italy. (Poster presentation)

2. **Li N**, van der Sijde MR, LifeLines Cohort Study, Bakker SJ, Dullaart RP, van der Harst P, Gansevoort RT, Wijmenga C, Snieder H, Hofker MH, Fu J. Polygenic Risk Models Suggest Protective Effects of Lipid Genes on Plasma Glucose, HbA1c and HOMA-IR levels". 36th European Lipoprotein Club meeting, September 9-12, 2013, Tutzing, Germany. (Poster presentation)

3. **N Li**, T Yuan, L Duan, M Li. Antibody-induced Severe Insulin Resistance Syndrome Successfully Diagnosed by Recovery Test. Annual Dutch Diabetes Research Meeting, November 29-30, 2013, Oosterbeek, the Netherlands. (Oral presentation)

4. **N Li**, R Chen, Y Zhu, L Xu, H Zhang, Y Li, K Zhang, M Li. Judgement of a patient of autoimmune hypoglycemia whose insulin autoantibody is pseudonegative. 14th International Congress of Endocrinology, March 26-30, 2010,

Kyoto, Japan. (Poster presentation)

5. **N Li**, W Li, H Wang. Intensively perioperative glycemic control with glargine in a group of perioperative patients with type 2 diabetic mellitus. 20th World Diabetes Congress, Oct 18-22, 2009, Montreal, Canada. (Poster presentation)

Abbreviations

BIP	Bezafibrate Infarction Prevention trial
CAD	coronary artery disease
CETP	cholesterol ester transfer protein
CLIA	Chemiluminescence immuno assays
CSII	continuous subcutaneous insulin infusion
DIDMOND	Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness
DPP	United States Diabetes Prevention Program
DPP- Ⅳ	Dipeptidyl Peptidase Ⅳ
DPS	Finland Diabetes Prevention Study
EBM	Evidence-based medicine
ELISA	Enzyme-linked immunosorbent assay
ER	endoplasmic reticulum
FPG	fasting plasma glucose
GLUT4	Glucose transporter type 4
GWAS	genome-wide association studies
HDL-C	high-density lipoprotein cholesterol
HGP	Human Genome Project
HOMA-IR	homeostatic model assessment-insulin resistance
IAS	Insulin autoimmune syndrome
IDPP	India Diabetes Prevention Program
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IGT	impaired glucose tolerance
IR	insulin resistance
LDL-C	low-density lipoprotein cholesterol
LPL	lipoprotein lipase
MDI	multiple dose injection
MODY	Maturity onset diabetes of the young
MR	Mendelian randomization
NAFLD	nonalcoholic fatty liver disease
NDM	Neonatal diabetes mellitus
NPH	Neutral protamine Hagedorn
OGTT	oral glucose tolerance test
PNDM	Permanent neonatal diabetes mellitus
PREVEND	Prevention of REnal and Vascular ENd stage Disease
PPAR	peroxisome proliferator-activated receptor
rHDL	reconstituted high density lipoproteins
RIA	Radioimmunoassay
SGLT2	sodium/glucose cotransporter 2
SOS	Swedish obese subjects study
T2D	type 2 diabetes
TC	total cholesterol
TG	triglycerides
UKPDS	United Kingdom Prospective Diabetes Study
ULSAM	Uppsala Longitudinal Study of Adult Men
VLDL	very low-density lipoprotein
WOSCOPS	West of Scotland Coronary Prevention Study