Bioinformatics of Metabolomics in Diabetes Mellitus Type 2

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ABSTRACT

Diabetes mellitus type 2 (DMT2) is a complex polygenic disorder. DMT2 is a result of insulin resistance and destruction of pancreatic β-cell or dysfunction. Therefore, glucose builds up in the bloodstream, leading to nerve damage, blindness, organ failures and sometimes death. Recently, some recently discovered genes play a key role in regulating the sensitivity to insulin. Scientists have long known that the disease often runs in families, and other genetic links. Human genetic discoveries will keep improving our knowledge about diabetes for many years to come. Varieties of prospective diabetic researches were developed to diagnose and control DMT2. Researchers spent thousands of millions of dollars to address DMT2. Pioneers of advanced biotechnology developed bioinformatics tools that changed the course of research about the role of metabolomics in DMT2. It will facilitate the identification of possible causes of DMT2 in genome studies. The present article aimed at reviewing the research studies per training to metabolomics and bioinformatics in genome studies in relation to DMT2.

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Introduction

Proteomics 2012 discussed a variety of proteomic and bioinformatic sciences ranging from computation methods, intracellular organelle proteomics, cellular proteomics, to clinical biological fluids/tissue proteomics and provided an excellent pathway for an effective multidisciplinary interactions in the control of diabetes[1].Omics methods such as proteomics and metabolomics, if used concurrently, can complement each other and provide an unbiased confirmation of biologically relevant signal pathways[2].

Globally, diabetes is one of the most common non-communicable metabolic disorders. It is the fourth or fifth leading cause of death in most high-income countries and there is substantial evidence to show that it is epidemic in many low- and middle-income countries. DM is a complex metabolic disease marked by the uncoupling of blood glucose levels and insulin secretion with causing abnormal glycemic stages. Complications from diabetes, such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure and blindness are resulting in increasing disability, reduced life expectancy and enormous health costs for virtually every society[3]. The complications guided to molecular investigations to study genes that are involved with both diseases and sets genes driving each illness separately. The identification of these different genetic backgrounds discloses a molecular link between DM and complications. DMT2 is marked by insulin resistance and pancreatic β-cell dysfunction, the latter possibly caused by a defect in insulin signaling in β-cells. In addition, the loss of insulin secretion may be attributed to loss of function in individual cells and loss of β-cell mass[4].

Abnormalities of metabolism (impaired fasting or postprandial glucose, dyslipidemia, etc.) early in life, markedly raises the risk of later in life evolving through modest abnormalities to full-blown disease with multi-organ complications. The earliest abnormalities are manifest as both insulin resistance and insufficient insulin secretion or a combination hereof[5]. Finding genes and gene clusters responsible for diabetes was little. The software tools available nowadays will enable scientists to pinpoint genetic variations or SNPs (Single Nucleotide Polymorphism) associated with type 2 diabetes.

Insulin resistance

Several studies showed that insulin resistance arises from disturbances in the ring communication between beta cells, liver, adipose tissue and skeletal muscle. These signaling defects affect insulin secretion and result in elevated blood glucose level[6]. Insulin resistance is marked by a diminished ability of insulin-sensitive tissues to take up and metabolize glucose in response to insulin leading to DMT2. Skeletal muscle is the primary site of insulin-mediated glucose disposal and contributes significantly to a decreased glucose uptake[7]. Insulin potentiates glucose-stimulated insulin secretion in insulin-resistant subjects to a lesser degree than in normal subjects. In target tissues, such as skeletal muscle, insulin promotes glucose uptake through the translocation of the glucose transporter from an intracellular vesicular pool to the plasma membrane[8-10].

Insulin receptor signaling has a central role in regulating cellular metabolism, growth, division, differentiation and survival. Insulin resistance contributes to the pathogenesis of DMT2[11]. Future research goals are to systematically identify the inter- and intra-cellular signaling pathways controlling cellular, whole body glucose and lipid homeostasis. Through the discovery of key regulatory proteins in glucose and energy homeostasis, new diabetes prevention and treatment targets can be identified and validated.

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In order to understand the genetic basis of DMT2, the genetic aspects of glucose metabolism in cells and their surrounding tissue need to be investigated. Researches include the innate, genetic macromolecules, such as nucleic acids (DNA and RNA) and their encoded proteins, and the acquired metabolic macromolecules[12].

**β-cell insulin secretion**

The endocrine pancreas comprises the islets of Langerhans, tiny clusters of cells that contribute only about 2% to the total pancreas mass. However, this little endocrine organ plays a critical role in maintaining glucose homeostasis by the regulated secretion of insulin, secreted by β cells and glucagon, secreted by α cells[13].

Multiple lines of evidence support the hypothesis that the pancreatic β-cell is an insulin-responsive tissue contributes to regulation of insulin secretion. Insulin receptors are present and functional in human β-cells [14]. In isolated human β-cells, insulin potentiates glucose-stimulated insulin secretion and de novo insulin synthesis. Human pancreatic islets from patients with DMT2 have reduced RNA expression of multiple insulin signaling proteins including the insulin receptor (IRS-2) and the serine/threonine protein kinase Akt2, which could impair insulin signal transduction and contribute to a diminished physiologic effect of insulin in patients with DMT2[15].

Advanced studies ensured that insulin secretion capacity reflects β cell activity. Therefore, it is an early and sensitive biomarker for β-cell activity. Many transcriptional factors control the development of β-cells and expression of many genes leading to impaired insulin secretion such as hepatocyte nuclear factor-4α, -1α, -1β (HNF-4α, -1α, -1β), insulin promoter factor-1 α (IPF-1α), and NEUROD1[16].

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**Genome analysis for DMT2 on-line database**

Developing systems biology approaches is to integrate comprehensive genetic information and provide new insight on DMT2 biology. Directed researches are required for identification and characterization of biological roles for genes regulating the blood glucose. A successful strategy was to screen relatively well-characterized patient groups for chromosomal “hot spots” and associated single nucleotide polymorphisms (SNPs). In DMT2 only approximately 28% of the disease heritability may be explained by identified individual SNPs that showed statistical significance in these samples/population a problem known as missing heritability [17-18].

Recently, conducted large-scales meta-analyses of GWAS (Genome Wide Association Studies) can improve our understanding of genetic variations and genetic risk factors involved in DMT2. These studies have identified DMT2 candidate as risk genes that are located in higher concentration in certain parts of the genome, specifically in chromosome 20, using the DMT2 genetic network[19]. Gene annotation information is derived from the VEGA (Vertebrate Genome Annotation database). A large amount of data available on the genetics of DMT2 from association studies of candidate gene variation were uploaded within gene banks data base[20].

In 2010, investigators explored a new technology to study genome-wide long-range chromatin interactions bound by protein factors with paired-end tag sequencing (ChIA-PET). ChIA-PET is a software package for automatic processing of ChIA-PET sequence data, including linker filtering, mapping tags to reference genomes, identifying protein binding sites and chromatin interactions, and displaying the results on a graphical

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A new web-based, Chromosome-Assembled human Proteome browser (CAPER) was designed to display proteomic data sets and related annotations. CAPER employs two distinct visualization strategies: track-view for the sequence/site information and the correspondence between proteome, transcriptome, genome, and chromosome and heat map-view for the qualitative and quantitative functional annotations. Both track-view and heat map-view can mutually switch, providing a high-quality user interface and facilitating the complete annotation and functional interpretation of the human genome by proteomic approaches (accessed at http://www.bprc.ac.cn/CAPE) [22].

The Gene Ontology Annotation (GOA) is a major bioinformatics and represented gene and gene product attributes across all species. Annotations created by the project are collated with annotations from external databases to provide an extensive, publicly available GO annotation resource at the EBI (European Bioinformatics Institute), ((http://www.ebi.ac.uk/goa)[23].

Several Human protein-protein interaction (PPI) databases have become available. Human Annotated and Predicted Protein Interaction (HAPPI) database, located at http://bio.informatics.iupui.edu/HAPPI/. The HAPPI database was created by extracting and integrating publicly available protein interaction databases, using database integration techniques. It enables its users to fully explore PPI data with quality measures and annotated information necessary for emerging network biology studies. It provides hyperlinked information of genes, pathways, protein domains, protein structure displays, and sequence feature maps for interactive exploration of PPI data in the database. The user can learn that this interaction as involved in several biological processes together, because the interacting proteins have several pathways such as insulin signaling in DMT2[24].

The University of California Santa Cruz (UCSC) Genome Browser is a popular Web-based tool for quickly displaying a requested portion of a genome at any scale. The annotations generated by the UCSC Genome Bioinformatics Group and external collaborators include gene predictions, mRNA and expressed sequence tag alignments, simple nucleotide polymorphisms, expression and regulatory data, phenotype and variation data, pair wise and multiple-species comparative genomics data. All information relevant to a region is presented in one window, facilitating biological analysis and interpretation. Users can upload personal datasets in a wide variety of formats as custom annotation tracks in both browsers for research or educational purposes [25].

**Benchmarking of β-cells gene expression for DMT2**

Screening each gene in the T2D linked region is necessary to prioritize positional genes located in the linked chromosomal regions that would facilitate and expedite the identification of disease genes.

**β-cell gene expression resources**

European experts in the field of DMT2 study monitored gene expression profiles of pancreatic β-cells and highlighted on genes and pathways involved in DMT2. The resulted database has built a unique collection of gene expression measurements performed on β-cells of human. The system integrates a web interface to several standard analysis functions from Bioconductor to identify differentially expressed genes and pathways. It allows rapid design for custom analysis.
pipeline and thus produces their own list of genes and pathways. The flexible engine of this database is currently used to handle gene expression data from several laboratory-run projects dealing with different organisms and platforms. The resource facilitates graphical presentation of the gene/genome wide map of SNP markers and protein-protein interaction networks, besides providing the heat map diagram of the selected gene(s) in an organism across microarray expression\[26\]-\[27\]. Several genome wide association studies added new facts to the knowledge on the molecular background of the complex form of DMT2. Scientists have substantially extended the short list of previously identified DMT2 genes as shown in table 2.

### Potential risk factors of DMT2

#### 1-Mitochondrial diabetes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Discovery method</th>
<th>Year</th>
<th>Presumed pathophysiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARg</td>
<td>3</td>
<td>Candidate gene approach</td>
<td>2000</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>KCNJ11</td>
<td>11</td>
<td>Candidate gene approach</td>
<td>2003</td>
<td>β-cell dysfunction</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>10</td>
<td>Genome wide linkage study</td>
<td>2006</td>
<td>β-cell dysfunction</td>
</tr>
<tr>
<td>CDKAL1</td>
<td>6</td>
<td>Genome wide association study</td>
<td>2007</td>
<td>β-cell dysfunction</td>
</tr>
<tr>
<td>CDKN2A/</td>
<td>9</td>
<td>Genome wide association study</td>
<td>2007</td>
<td>β-cell dysfunction</td>
</tr>
<tr>
<td>FTO</td>
<td>16</td>
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<td>2007</td>
<td>Obesity</td>
</tr>
<tr>
<td>HHEX/IDE</td>
<td>10</td>
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<td>2007</td>
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<tr>
<td>IGF2BP2</td>
<td>3</td>
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<tr>
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<td>β-cell dysfunction</td>
</tr>
<tr>
<td>WFS1</td>
<td>4</td>
<td>Genome wide association study</td>
<td>2007</td>
<td>β-cell dysfunction</td>
</tr>
</tbody>
</table>
The mitochondrial genome is a circle, 16.6 kb of DNA with 2-4 Mbp. Mitochondrial DNA (mtDNA) abnormalities are known to cause insulin deficiency, insulin resistance and diabetes mellitus. From a population based epidemiologic study, it was found that mtDNA quantity was decreased in the peripheral blood of diabetic subjects, and also in those subjects who will convert to diabetes mellitus within 2 years[28-30]. Functional defects in adipocytes may cause systemic disturbance of glucose homeostasis. Recent studies revealed mitochondrial abnormalities in the adipose tissue of patients with DMT2. Besides, patients with mitochondrial diseases usually manifest systemic metabolic disorder. However, it is unclear as to how mitochondrial dysfunction in adipocytes affects the regulation of glucose homeostasis[31].

Some researchers explored an attenuation of the insulin response as indicated by lower glucose uptake and decreased phosphorylation upon insulin stimulation of adipocytes with mitochondrial dysfunction. Besides, the expression of glucose transporter 4 (Glut4) and secretion of adiponectin were decreased in adipocytes with increased reactive oxygen species generated by defective mitochondria. Moreover, the severity of insulin insensitivity was correlated with the extent of mitochondrial dysfunction. These results suggest that higher intracellular ROS (Reactive Oxygen Species) levels elicited by mitochondrial dysfunction resulted in impairment of the function of adipocytes in the maintenance of glucose homeostasis through attenuation of insulin signaling, down-regulation of Glut4 expression, and decrease in adiponectin secretion[32-33]. The mitochondria has important role in regulation of glucose homeostasis in adipocytes. A molecular basis is required for the explanation of manifestation of diabetes mellitus or insulin insensitivity in a portion of patients with mitochondrial diseases.

2-Glucokinase gene

Glucokinase (GCK) gene encodes an enzyme of the glycolytic pathway "Glucokinase". GCK is expressed in pancreatic beta cells, where it may play a key role in sensing plasma glucose to insulin release. GCK is also expressed in hepatocytes, where the phosphorylation of glucose by GCK may be essential to hepatic glucose disposal. The GCK gene has been characterized as containing 12 exons. GCK mutation, as yet unidentified genetic modifiers, and/or environmental factors might have different effects on pancreatic beta-cell functions, causing variability in the age at diagnosis of diabetes. Testing for Maturity-onset diabetes of the young type 2 and reporting all novel mutations are important to avoid difficulties in the interpretation of genetic test results and to provide fast and definitive diagnosis for all patients with this disease[34-35].

Patients with GCK mutations show impaired insulin secretion, that in turn are possibly at risk for developing diabetes mellitus by triggering factors. A defect of glucokinase gene is one of the candidates for the pathogenesis of noninsulin-dependent diabetes mellitus. Recently, a close linkage of noninsulin-dependent diabetes mellitus to the glucokinase gene was found in maturity-onset diabetes of the young (MODY) patients, and a nonsense mutation of exon 7 of glucokinase gene was reported in one of these pedigrees[36-37].

3- Vitamin D and metabolomics in DMT2

It is virtual that, genes encoding proteins participating in vitamin D metabolism may influence susceptibility to DMT2. This applies to proteins involved in the formation of active forms of vitamin D, its transportation in body fluids, receptor action and post-receptor effectors. Several studies reported that vitamin D deficiency is regarded as a potential risk factor for DMT2. It is involved in the pathogenesis of pancreatic β-cell dysfunction, insulin resistance and systemic inflammation, conditions that contribute to the
development of DMT2. It can affect the progress of this disease directly by activation of its own receptor and indirectly via the regulation of calcium homeostasis [38-39].

One of those genes is vitamin D binding protein (DBP). The DBP sequence differences have two frequent amino acid variants: GAT to GAG substitution at codon 416 that causes replacement of aspartic acid by glutamic acid and ACG to AAG substitution at codon 420 that changes threonine to lysine. It was shown that they (or corresponding electrophoretic variants of DBP glycoprotein) influenced quantitative pre-diabetic traits in several populations, including Pima Indians, the ethnic group with a very high prevalence of DMT2[40]. Vitamin D may not be effective enough to improve insulin resistance and related morbidities. Therefore, they should ideally receive further nutritional support according to their genotype[41].

4 - Oxidative Stress

Oxidative stress may contribute to the development of DM. mainly, oxidative stress is due to variation in the genes coding these enzymes endogenous antioxidants including antioxidant enzymes e.g. superoxide dismutase, catalase, glutathione peroxidase, paraoxanase, and glutathione S - transferase. Many recent nutrigenetics studies highlighted relation of interactions between diet, genetic variation in antioxidant enzymes, and oxidative stress[42-43]. Indeed, polymorphisms in most of the genes that code for antioxidant enzymes have been associated with several types of cancer, although inconsistent findings between studies have been reported. These inconsistencies may, in part, be explained by interactions with the environment, such as modification by diet.

5- Maturity-onset diabetes of the young (MODY)

MODY is caused by an inactivating mutation in the gene for glucokinase (GCK), leading to defective glucose stimulated insulin secretion. It demonstrates differential glycemic/C-peptide responses to treatment with insulin, no medication, and an oral sulfonylurea. A genetic analysis revealed a novel GCK mutation (c.46-15_46-11del nsGGGAGGG) in intron 1 of the CGK gene. In future, different GCK mutations and biochemical analysis of the patient’s biological parents require evaluation of their genotype/phenotype relationship[44-46].

Discussion:

DMT2 is a major cause of morbidity and mortality and represents a significant economic burden on health systems all over the world. The use of bioinformatics data base of the genome studies can change the course of research exploring the linkages between β cells and the potential risk factors for DMT2 as well as investigating the genes controlling insulin signaling pathways. The molecular basis of insulin insensitivity can be understood with the help of metabolomics to explain manifestations of DMT2, to predict disease onset and to reveal new biomarkers of the disorder. Currently, despite its potential benefits, genetic testing is considered by many healthcare professionals to be unnecessary or too expensive to be widely used. The solution may be the incorporation of non-genetic biomarkers and bioinformatics into genetic research to render the screening molecular diagnosis of disorders like DMT2 less expensive. Such testing could be possibly combined with phenotypic, clinical or laboratory investigation. The data from the Genome project can be utilized to identify novel DMT2 genes involved in the cross-linkages between insulin signaling systems and metabolomics.

Summary:

Understand genetic basis of DMT2 and how mutations may affect human health require studying the
genetic view of glucose metabolism in cells and their surrounding tissue. Researches include the innate, genetic macromolecules, such as nucleic acids and their encoded proteins, and the acquired metabolic macromolecules.

Large-scale Genome meta-analyses results improved interpretations of genetic variations and genetic risk factors. Such research determined DMT2 candidate as risk genes that are located in higher concentration in certain parts of the genome, specifically in chromosome 20, using the DMT2 genetic network. Scientists have substantially extended the short list of previously identified DMT2 genes.

Several genome wide association studies added new facts to the knowledge on the molecular background of the complex form of DMT2. The mitochondria has an important role in regulation of glucose homeostasis in adipocytes. Genes encoding proteins participating in vitamin D metabolism may influence susceptibility to DMT2. Patients with GCK mutations show impaired insulin secretion, that in turn are possibly at risk for developing diabetes mellitus by triggering factors. Oxidative stress may contribute to the development of DM due to variation in the genes coding these enzymes of endogenous antioxidants including antioxidant enzymes.

Conclusion:

Genome-wide association data mining for identifying novel DMT2 genes are involved in the crosstalk between insulin signaling systems and metabolomics especially potential risk factors. The data from the Genome project can be utilized to identify novel DMT2 genes involved in the cross-linkages between insulin signaling systems and metabolomics.

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References


18. Lim JE, Hong KW, Jin HS, Kim YS, Park HK, Oh B: Type 2 diabetes genetic association database manually curated for the study design and odds ratio. BMC Med Inform DecisMak 2010; 10:76.


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