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MicroRNA Microarray Analysis Combined with Interaction Network Analysis to Investigate the Influence of Clozapine to Metabolic Syndrome

[1,2]Jianhua Chen, [1]Lin Yang, [1]Dengtang Liu, [1]Donghong Cui, [1]Shunying Yu, [1]Yan Li, [1]Haisu Wu, [3]Ying Yue, [2,4]Yongyong Shi and [1]Yifeng Xu
[1]Schizophrenia Program, Shanghai Mental Health Center, School of Medicine, Shanghai Jiao Tong University, 200030 Shanghai, China
[2]Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Bio-X Institutes (Ministry of Education), Shanghai Jiao Tong University, 200030, Shanghai, People's Republic of China
[3]Shanghai Luwan Mental Health Center, 1162 Quxi Road, 200023, Shanghai, People's Republic of China
[4]Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, 200042, Shanghai, People's Republic of China

Abstract: The Metabolic Syndrome (MetS) could significantly increase the risk of morbidity and mortality from type II diabetes and cardiovascular disease. We carried out a systemical study to investigate the potential drug targets for effective treatment of clozapine-induced MetS. In our study, the differentially-expressed miRNAs (DERs) between schizophrenia patients with MetS and without MetS after the treatment of clozapine were identified. Target genes of these miRNAs were then retrieved from two miRNA databases (miRecods and miRTarBase) to identify the underlying mechanisms involved in the development of MetS. Interactors of the target genes were identified and a network was constructed using Osprey. Functional enrichment analysis was performed for all the genes in the network with DAVID (Database for Annotation, Visualization and Integrated Discovery). The results revealed that 3 DERs including Hsa-miR-330-3p ($p = 0.00934$), has-miR-18a ($p = 0.001686$) and has-miR-106b ($p = 0.007531$)were identified. In total, 4 target genes (E2F1, CTGF, ESR1 and ITCH) shared by both databases were picked out. What's more, 8 significant pathways were revealed in the interaction network and the most significant pathway was positive regulation of macromolecule metabolic process (FDR = 1.80E-15). In conclusion, the 3 identified miRNAs might be the potential targets for treatment of MetS. And the results of our study provide ways to monitor the progression of MetS (e.g., serum levels of proteins), predict the outcome (e.g., polymorphisms) and even block the emergence of MetS.

Key words: Metabolic syndrome, differentially expressed miRNA, target gene, interaction network, functional annotation enrichment analysis

INTRODUCTION

The metabolic syndrome (MetS) is a clustering of metabolic, anthropometric and haemodynamic abnormalities that, when occurring together, significantly increase the risk of morbidity and mortality from type 2 diabetes and cardiovascular disease (CVD). The features which include hyperglycemia, central obesity, hyperlipidemia and hypertension may share a similar pathogenesis and in combination they pose more damage to the heart, brain, kidneys and other vital organs than simple high blood pressure or diabetes. For example, the risk of cerebrovascular disease is higher for patients with MetS compared with the patients without MetS and mortality is 5-6 times higher (Eckel *et al*., 2005). In fact, MetS-induced cardiovascular and cerebrovascular disease, myocardial infarction and pulmonary diseases are expected to become the three leading causes of death in the future (Mottillo *et al*., 2010).

One possible antecedent of MetS in psychiatric settings is the mainstream pharmacological treatment of psychotic disorders (e.g., schizophrenia) and severe behavioral disorders. Although psychotropic drugs are necessary to treat these disorders, among the side effects are an increased incidence of cardiovascular disease (Anonymous, 2004a), diabetes (De Hert *et al*., 2008) and weight gain, abnormal glucose and lipid metabolism (De Hert *et al*., 2009; McEvoy *et al*., 2005; Pramyothin and

Corresponding Author: Yifeng Xu, Shanghai Mental Health Center, Shanghai Jiao Tong University, School of Medicine, 600 Wan Ping Nan Road, 20030, Shanghai, China
Yongyong Shi, Bio-X Institutes, Shanghai Jiao Tong University, Central Little White House, 1954 Huashan Road, 200030, Shanghai, China

Khaodhiar, 2010; Walss-Bass *et al*., 2008). As the results, patients with schizophrenia who are subjected to long-term use of psychotropic drugs, such as clozapine and olanzapine, are at risk of and suffer from a higher incidence of MetS than general population (Anonymous, 2004b).

Many studies involving patients with clozapine-induced MetS have been conducted, but most of the studies have had a limited focus. For example, (Mulder *et al*., 2009) explored the association between 5-hydroxytryptamine (serotonin) receptor 2C polymorphisms and MetS in patients with schizophrenia (Mulder *et al*., 2009). Other investigations have explored the polymorphisms of leptin and its receptor (Boumaiza *et al*., 2012), pyruvate dehydrogenase kinase 4 (Moon *et al*., 2012), fibroblast growth factor 21 (Zhang *et al*., 2012), paraoxonase-1 (Kordi-Tamandani *et al*., 2012) and so on among this patient group. Expression levels of critical genes have also taken investigated. Gormez *et al*. (2011) suggested that pathogenesis of MetS was associated with the expression levels of adiponectin, necrosis factor alpha and leptin (Gormez *et al*., 2011). However, it is likely that there are global changes in gene expression in patients with schizophrenia with clozapine-induced MetS and that these changes cannot be explained by simply one means. Therefore, Grayson and others have focused on the gene expression profile of peripheral blood (Grayson *et al*., 2011) and Bahr and others collected mRNA profiles of adipose tissue from rats (Bahr *et al*., 2011). Additionally, a range of tissues have been studied, including pancreatic islets (Dreja *et al*., 2010) and mononuclear cells (Camargo *et al*., 2010). Though considerable data have been obtained, the understanding of the progression of MetS is not yet suffciently adequate to inform clinical interventions.

A global description of the changes in MetS including upstream regulatory factors (such as miRNAs and transcriptional factors) and downstream "effector" is beneficial. In recent years, miRNAs have been identified as key regulators of gene expression (Cannell *et al*., 2008) and have been implicated in many metabolic process (Rottiers and Naar, 2012), such as lipid metabolism (Sacco and Adeli, 2012) and vascular biology (Schober *et al*., 2012). And these findings could be useful in helping us to understand the pathogenesis of MetS.

The current study aimed to systemically investigate the changes and position potential drug targets for effective treatment of clozapine-induced MetS. We analyzed miRNA chip data to identify differentially-expressed miRNAs (DERs). Target genes of the DERs were retrieved and functional enrichment analysis as well as interaction network analysis were conducted to identify any alterations in gene expression level.

MATERIALS AND METHODS

Subjects: Our sample consisted of 24 unrelated patients (12 males and 12 females) who had been diagnosed with schizophrenia according to DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) by psychiatrists at the Shanghai Mental Health Center in China. All patients were Han Chinese and provided their informed consent to participate in the study, which had been approved by the local research ethics committee.

All twenty-four patients had been taking clozapine for more than one year.

Criteria for MetS: The status of MetS for each patient was established using the IDF (International Diabetes Federation) criteria (Alberti *et al*., 2005). Those with central obesity assessed by waist circumference (Chinese≥90 cm for males and ≥80 cm for females) and have any two or more of the following factors: Elevated concentration of triglycerides (>150 mg dL^{-1}), reduced concerntration of high density lipoprotein cholesterol (<40 mg dL^{-1} in men and <50 mg dL^{-1} in women), raised fasting glucose concentration (≥100 mg dL^{-1}) and raised systolic arterial blood pressure (≥130 mmHg) and/or diastolic arterial blood pressure (≥85 mmHg) were identified as having MetS.

The mean age of patients with MetS was 46.4±5.0 years and the mean age of patients without MetS subjects was 44.4±7.1 years.

MiRNA microarray: We collected peripheral blood samples. Every four samples were pooled and analyzed with one Affymetrix micro RNA 2.0 chip. A total of 6 chip data were obtained with 3 normal metabolism and 3 abnormal metabolism.

Package Affy (Fujita *et al*., 2006; Troyanskaya *et al*., 2001) of R was used to convert original CEL format into expression profile format and then normalize the data with median method. Package multtest (Dudoit *et al*., 2003) was chosen for differential analysis with the t-test method. A p value of less than 0.01 was set as the significance cut-off criterion.

Retrieval of target genes: Target genes of the DERs were retrieved from two miRNA databases: MiRecords and miRTarBase, respectively. Both databases gather

experimentally verified target genes. The miRecords database contains 548 miRNAs and corresponding target genes from 9 species (Xiao *et al*., 2009), while the miRTarBase database includes 773 miRNAs and 2632 target genes from 14 species (Hsu *et al*., 2011). Since each miRNA has a range of target genes based upon different algorithms, those shared by both databases were regarded as of high confidence and were retained for the analyses.

Interaction network analysis: Osprey (Breitkreutz *et al*., 2003a) was adopted to retrieve interactors of the target genes and construct the interaction network. This network visualisation system was developed for use in studies about protein-protein interaction networks and protein complexes. Osprey currently contains more than 50,000 interactions and it is connected with the BIND (Biomolecular Interaction Network Database) (Willis and Hogue, 2006) and GRID (Global Resource Information Database) (Breitkreutz *et al*., 2003b).

DAVID (Database for Annotation, Visualization and Integrated Discovery) (Huang *et al*., 2009), a clustering tool based on the hypergeometric distribution, was chosen as the analytic tool in our study. An FDR (false discovery rate) value of less than 0.05 was set as the cutoff criterion.

RESULTS AND DISCUSSION

Identification of DERs: A good performance of normalization was achieved (Fig. 1). Differential analysis was performed using t-test method from package multtest between 3 chips with abnormal metabolism and 3 with normal metabolism. A total of 3 miRNAs were identified: Hsa-of miR-330-3p ($p = 0.00934$), has-of miR-18a ($p = 0.001686$) and has-of miR-106b ($p = 0.007531$).

Target genes for DERs: Target genes of the three DERs were retrieved through searching within miRecords and miRTarBase, individually. Then four genes shared by both databases were picked out for further analysis: E2F1, CTGF, ESR1 and ITCH (Table 1).

Interaction network for target genes: Interactors of the four target genes were acquired and the network consisting of 53 nodes was constructed with Osprey (Fig. 2). Database for Annotation, Visualization and Integrated Discovery was used to perform functional enrichment analysis for all the genes in the network and 8 significant pathways were uncovered (Table 2). The most significant pathway was positive regulation of macromolecule metabolic process (FDR = 1.70E-15), while others were also associated with regulation of metabolic process, such as RNA and DNA.

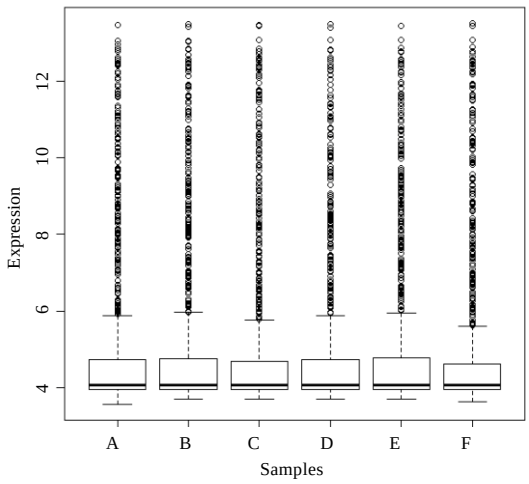

Fig. 1: Box plot for normalized gene expression data. A, B and C represent the three samples with normal metabolism while D, E and F are the three samples with abnormal metabolism. Black lines in the boxes indicate the medians. The medians are almost on the same line, suggesting a good performance of the normalization

Table 1: Target genes retrieved from the two databases

	miRTarBase		miRecords	
ID	Target gene	References (PMID)	Target gene_name	Pubmed_id
Hsa-miR-330-3p	E2F1	19597470	E2F1	19597470
Hsa-miR-18a	CTGF	16331254	CTGF	20305691
Hsa-miR-18a	ESR1	19706389	ESR1	20080637
Hsa-miR-106b	E2F1	18676839	E2F1	19486339
Hsa-miR-106b	ITCH	19074548	ITCH	19096009

Table 2: Over-represented biological pathways for all genes in the network

Term	Function	FDR
GO:0010604	positive regulation of macromolecule metabolic process	1.70E-15
GO:0051173	positive regulation of nitrogen compound metabolic process	1.79E-12
GO:0051254	positive regulation of RNA metabolic process	5.75E-07
GO:0051252	regulation of RNA metabolic process	1.16E-06
GO:0006259	DNA metabolic process	1.05E-04
GO:0051174	regulation of phosphorus metabolic process	5.52E-04
GO:0019220	regulation of phosphate metabolic process	5.52E-04
GO:0051247	positive regulation of protein metabolic process	7.11E-04

It could be speculated that psychotropic drugs primarily caused abnormal expression of hsa-miR-330-3p, has-miR-18a and has-miR-106b which affected target genes and their interactors that were implicated in regulation of biomolecule metabolic process.

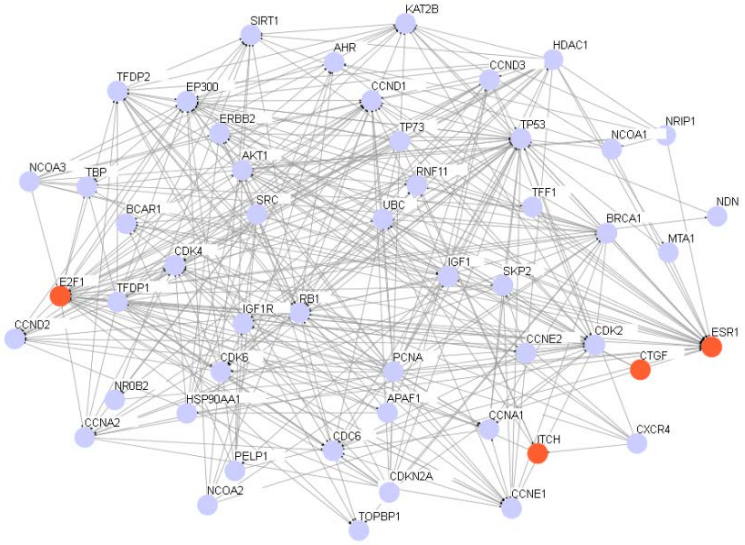

Fig. 2: Interaction network for target genes generated by Osprey. Red circles represent the four verified target genes and the gray circles are the interactors of the target genes

We applied miRNA microarray technology to determine the global expression changes for patients with MetS compared with those without MetS. A total of 3 differentially expressed miRNAs were identified: Hsa-miR-330-3p, has-miR-18a and has-miR-106b.

E2F transcription factor 1 (E2F1) is the target of Hsa-miR-330-3p (Emmrich and Putzer, 2010; Lee *et al*., 2009). E2F1 is a member of the E2F family of transcription factors which play a critical role in the control of cell cycle and action of tumor suppressor proteins. E2F1 also takes a part in metabolic processes (Fajas and Annicotte, 2011). E2F1 regulates the expression level of pyruvate dehydrogenase kinase 4 and thus influences the metabolism in human cardiac cells (Palomer *et al*., 2011). Blanchet *et al*. (2009) indicate that the CDK4-pRB-E2F1 regulatory pathway is involved in general glucose homeostasis and metabolism (Blanchet *et al*., 2009).

Perri *et al*. (2012) investigate the expression of miRNAs in obese patients and report that miR-18a is up-regulated in patients with obesity while miR-106b is up-regulated in non-obese individuals (Perri *et al*., 2012). The target of miR-18a contains connective tissue growth factor (CTGF) (Ohgawara *et al*., 2009) and estrogen receptor 1 (ESR1) (Loven *et al*., 2010). CTGF is a mitogen that is secreted by vascular endothelial cells. The encoded protein plays a role in chondrocyte proliferation and differentiation, cell adhesion in many cell types and is related to platelet-derived growth factors. Colak and others report that serum levels of CTGF may be of clinical utility for distinguishing nonalcoholic fatty liver disease patients with and without advanced fibrosis (Colak *et al*., 2012). The positive role of CTGF in the development of cardiac hypertrophy and fibrosis has also been demonstrated (Yoon *et al*., 2010). ESR1 is an estrogen receptor and also a ligand-activated transcription factor. The relationship between its polymorphisms and lipid metabolism has been extensively studied (Molvarec *et al*., 2007; Sertic *et al*., 2009).

The miR-106b is associated with cholesterol metabolism. Kim *et al*. (2012) indicate that miR-106b impairs cellular cholesterol efflux and increases amyloid β level by repressing ATP-binding cassette transporter A1 expression (Kim *et al*., 2012). Its target includes E2F1 (Trompeter *et al*., 2011) and itchy E3 ubiquitin protein ligase (ITCH) (Rivetti di Val Cervo *et al*., 2009). ITCH is a member of the Nedd4 family of HECT domain E3 ubiquitin ligases and plays a role in multiple cellular processes.

To further confirm the regulation role of these miRNAs in MetS, the interaction network was established for the four target genes using Osprey. Functional annotation enrichment analysis was then performed with DAVID for all the genes in the network and 8 significant annotations were revealed (Table 2). In functional enrichment analysis, a group of genes sharing similar or relevant functions are considered as a whole which greatly reduces the dimensions of the data analysis and facilitates the determination of changes in biological processes. Therefore, it is widely adopted in analysis of chip data (Huang *et al*., 2008).

All the biological pathways were associated with metabolic process. The most significantly over-represented one was positive regulation of macromolecule metabolic process, while metabolisms of phosphate and nitrogen compound were also significant. The linkages between some interactors and MetS have been reported. It has been found that v-akt murine thymoma viral oncogene homolog 1 (AKT1) is associated with glucose homeostasis and MetS (Devaney *et al*., 2011; Harmon *et al*., 2010). The polymorphism of insulin-like growth factor 1 receptor (IGF1R) is also linked to MetS (Kilpelainen *et al*., 2008). Tumor protein p53 (TP53) is also a regulator in lipid metabolism pathways (Goldstein *et al*., 2012). Further, IGF1 (Hu *et al*., 2010) and cyclin-dependent kinase 4 (CDK4) (Blanchet *et al*., 2011) are also involved in the regulation of metabolic process. Taken together, the interaction network of the target genes is involved in the regulation of various metabolic processes which validates the roles for the 3 miRNAs.

CONCLUSION

In summary, we integrated miRNA microarray analysis and interaction network analysis to identify miRNAs implicated in the regulation of metabolic process. It is likely that clozapine influences the expression pattern of gene through the regulation of miRNAs and thus results in abnormalities of metabolism which increases the risk of metabolic syndrome. Three miRNAs could be potential targets for treatment of MetS. In addition, many relevant genes were also closely related to MetS which provides ways to monitor the progression of MetS (e.g., serum levels of proteins), predict the outcome (e.g,. polymorphisms) and even block the emergence of MetS.

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REFERENCES

Alberti, K.G.M.M., P. Zimmet and J. Shaw, 2005. The metabolic syndrome-a new worldwide definition. Lancet, 366: 1059-1062.

Anonymous, 2004a. Consensus development conference on antipsychotic drugs and obesity and diabetes. J. Clin. Psychiatry, 65: 267-272.

Anonymous, 2004b. Schizophrenia and Diabetes 2003 Expert Schizophrenia and Diabetes 2003 Expert Consensus Meeting, Dublin, 3-4 October 2003: Consensus summary. Br. J. Psychiat., Suppl 47 184: S112-S114.

Bahr, J., N. Kloting, I. Kloting and N. Follak, 2011. Gene expression profiling supports the role of Repin1 in the pathophysiology of metabolic syndrome. Endocrine, 40: 310-314.

Blanchet, E., J.S. Annicotte and L. Fajas, 2009. Cell cycle regulators in the control of metabolism. Cell cycle, 8: 4029-4031.

Blanchet, E., J.S. Annicotte, S. Lagarrigue, V. Aguilar and C. Clape *et al*., 2011. E2F transcription factor-1 regulates oxidative metabolism. Nat. Cell Biol., 13: 1146-1152.

Boumaiza, I., A. Omezzine, J. Rejeb, L. Rebhi and A. Ouedrani *et al*., 2012. Relationship between leptin G2548A and leptin receptor Q223R gene polymorphisms and obesity and metabolic syndrome risk in *Tunisian volunteers*. Genet. Test. Mol. Biomarkers, 16: 726-733.

Breitkreutz, B.J., C. Stark and M. Tyers, 2003a. Osprey: A network visualization system. Genome Biol., 10.1186/gb-2003-4-3-r22

Breitkreutz, B.J., C. Stark and M. Tyers, 2003b. The GRID: The general repository for interaction datasets. Genome Biol.,

Camargo, A., J. Ruano, J.M. Fernandez, L.D. Parnell and A. Jimenez *et al*., 2010. Gene expression changes in mononuclear cells in patients with metabolic syndrome after acute intake of phenol-rich virgin olive oil. BMC Genomics, Vol. 11,

Cannell, I.G., Y.W. Kong and M. Bushell, 2008. How do microRNAs regulate gene expression? Biochem. Soc. Trans., 36: 1224-1231.

Colak, Y., E. Senates, E. Coskunpinar, Y.M. Oltulu and E. Zemheri *et al*., 2012. Concentrations of connective tissue growth factor in patients with nonalcoholic fatty liver disease: Association with liver fibrosis. Dis. Markers, 33: 77-83.

De Hert, M., V. Schreurs, D. Vancampfort and R. van Winkel, 2009. Metabolic syndrome in people with schizophrenia: A review. World Psychiatry, 8: 15-22.

De Hert, M., V. Schreurs, K. Sweers, D. van Eyck and L. Hanssens *et al*., 2008. Typical and atypical antipsychotics differentially affect long-term incidence rates of the metabolic syndrome in first-episode patients with schizophrenia: A retrospective chart review. Schizophr. Res., 101: 295-303.

Devaney, J.M., H. Gordish-Dressman, B.T. Harmon, M.K. Bradbury and S.A. Devaney *et al*., 2011. AKT1 polymorphisms are associated with risk for metabolic syndrome. Hum. Genet., 129: 129-139.

Dreja, T., Z. Jovanovic, A. Rasche, R. Kluge and R. Herwig *et al*., 2010. Diet-induced gene expression of isolated pancreatic islets from a polygenic mouse model of the metabolic syndrome. Diabetologia, 53: 309-320.

Dudoit, S., J.P. Shaffer and J.C. Boldrick, 2003. Multiple hypothesis testing in microarray experiments. Stat. Sci., 18: 71-103.

Eckel, R.H., S.M. Grundy and P.Z. Zimmet, 2005. The metabolic syndrome. Lancet, 365: 1415-1428.

Emmrich, S. and B.M. Putzer, 2010. Checks and balances: E2F-microRNA crosstalk in cancer control. Cell Cycle, 9: 2555-2567.

Fajas, L. and J.S. Annicotte, 2011. E2F1 at the crossroad of proliferation and oxidative metabolism. Cell Cycle, 10: 4193-4194.

Fujita, A., J.R. Sato, L. de Oliveira Rodrigues, C.E. Ferreira and M.C. Sogayar, 2006. Evaluating different methods of microarray data normalization. BMC Bioinformatics, Vol. 7. 10.1186/1471-2105-7-469

Goldstein, I., O. Ezra, N. Rivlin, A. Molchadsky, S. Madar, N. Goldfinger and V. Rotter, 2012. p53, a novel regulator of lipid metabolism pathways. J. Hepatol., 56: 656-662.

Gormez, S., A. Demirkan, F. Atalar, B. Caynak and R. Erdim *et al*., 2011. Adipose tissue gene expression of adiponectin, tumor necrosis factor-α and leptin in metabolic syndrome patients with coronary artery disease. Int. Med., 50: 805-810.

Grayson, B.L., L. Wang and T.M. Aune, 2011. Peripheral blood gene expression profiles in metabolic syndrome, coronary artery disease and type 2 diabetes. Genes Immun., 12: 341-351.

Harmon, B.T., S.A. Devaney, H. Gordish-Dressman, E.K. Reeves, P. Zhao, J.M. Devaney and E.P. Hoffman, 2010. Functional characterization of a haplotype in the AKT1 gene associated with glucose homeostasis and metabolic syndrome. Hum. Genet., 128: 635-645.

Hsu, S.D., F.M. Lin, W.Y. Wu, C. Liang and W. Huang *et al*., 2011. miRTarBase: A database curates experimentally validated microRNA-target interactions. Nucleic Acids Res., 39: D163-169.

Hu, C., R. Zhang, C. Wang, J. Wang and X. Ma *et al*., 2010. Variants from GIPR, TCF7L2, DGKB, MADD, CRY2, GLIS3, PROX1, SLC30A8 and IGF1 are associated with glucose metabolism in the Chinese. PLoS One, Vol. 5,

Huang, D.W., B.T. Sherman and R.A. Lempicki, 2008. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protocols, 4: 44-57.

Huang, D.W., B.T. Sherman and R.A. Lempicki, 2009. Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res., 37: 1-13.

Kilpelainen, T. O., T.A. Lakka, D.E. Laaksonen, U. Mager and T. Salopuro *et al*., 2008. Interaction of single nucleotide polymorphisms in ADRB2, ADRB3, TNF, IL6, IGF1R, LIPC, LEPR and GHRL with physical activity on the risk of type 2 diabetes mellitus and changes in characteristics of the metabolic syndrome: The finnish diabetes prevention study. Metabolism, 57: 428-436.

Kim, J., H. Yoon, C.M. Ramirez, S.M. Lee, H.S. Hoe, C. Fernandez-Hernando and J. Kim, 2012. MiR-106b impairs cholesterol efflux and increases Aα levels by repressing ABCA1 expression. Exp. Neurol., 235: 476-483.

Kordi-Tamandani, D.M., M. Hashemi, N. Sharifi, M.A. Kaykhaei and A. Torkamanzehi, 2012. Association between paraoxonase-1 gene polymorphisms and risk of metabolic syndrome. Mol. Biol. Rep., 39: 937-943.

Lee, K.H., Y.L. Chen, S.D. Yeh, M. Hsiao, J.T. Lin, Y.G. Goan and P.J. Lu, 2009. MicroRNA-330 acts as tumor suppressor and induces apoptosis of prostate cancer cells through E2F1-mediated suppression of Akt phosphorylationmiR-330 regulates E2F1-Akt signaling of apoptosis. Oncogene, 28: 3360-3670.

Loven, J., N. Zinin, T. Wahlstrom, I. Muller and P. Brodin *et al*., 2010. MYCN-regulated microRNAs repress estrogen receptor-α (ESR1) expression and neuronal differentiation in human neuroblastoma. Proc. Natl. Acad. Sci. USA, 107: 1553-1558.

McEvoy, J.P., J.M. Meyer, D.C. Goff, H.A. Nasrallah and S.M. Davis *et al*., 2005. Prevalence of the metabolic syndrome in patients with schizophrenia: Baseline results from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) schizophrenia trial and comparison with national estimates from NHANES III. Schizophr. Res., 80: 19-32.

Molvarec, A., B. Nagy, M. Kovacs, S. Walentin and E. Imreh *et al*., 2007. Lipid, haemostatic and inflammatory variables in relation to the estrogen receptor alpha (ESR1) PvuII and XbaI gene polymorphisms. Clinica Chimica Acta, 380: 157-164.

Moon, S.S., J.E. Lee, Y.S. Lee, S.W. Kim, N.H. Jeoung, I.K. Lee and J.G. Kim, 2012. Association of pyruvate dehydrogenase kinase 4 gene polymorphisms with type 2 diabetes and metabolic syndrome. Diabetes Res. Clin. Pract., 95: 230-236.

Mottillo, S., K.B. Filion, J. Genest, L. Joseph and L. Pilote *et al*., 2010. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J. Am. Coll. Cardiol., 56: 1113-1132.

Mulder, H., D. Cohen, H. Scheffer, C. Gispen-de Wied and J. Arends *et al*., 2009. HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia: A replication study. J. Clin. Psychopharmacol., 29: 16-20.

Ohgawara, T., S. Kubota, H. Kawaki, S. Kondo and T. Eguchi *et al*., 2009. Regulation of chondrocytic phenotype by micro RNA 18a: Involvement of Ccn2/Ctgf as a major target gene.v FEBS Lett., 583: 1006-1010.

Palomer, X., D. Alvarez-Guardia, M.M. Davidson, T.O. Chan, A.M. Feldman and M. Vazquez-Carrera, 2011. The interplay between NF-kappaB and E2F1 coordinately regulates inflammation and metabolism in human cardiac cells. PLoS One, Vol. 6

Perri, R., S. Nares, S. Zhang, S.P. Barros and S. Offenbacher, 2012. MicroRNA modulation in obesity and periodontitis. J. Dent. Res., 91: 33-38.

Pramyothin, P. and L. Khaodhiar, 2010. Metabolic syndrome with the atypical antipsychotics. Curr. Opin. Endocrinol. Diabetes Obes., 17: 460-466.

Rivetti di Val Cervo, P., P. Tucci, A. Majid, A.M. Lena and M. Agostini *et al*., 2009. P73, miR106b, miR34a and Itch in chronic lymphocytic leukemia. Blood, 113: 6498-6499.

Rottiers, V. and A.M. Naar, 2012. MicroRNAs in metabolism and metabolic disorders. Nat. Rev. Mol. Cell Biol., 13: 239-250.

Sacco, J. and K. Adeli, 2012. MicroRNAs: Emerging roles in lipid and lipoprotein metabolism. Curr. Opin. Lipidol., 23: 220-225.

Schober, A., T. Thum and A. Zernecke, 2012. MicroRNAs in vascular biology-metabolism and atherosclerosis. Thromb. Haemost., 107: 603-604.

Sertic, J., L. Juricic, H. Ljubic, T. Bozina and J. Lovric *et al*., 2009. Variants of ESR1, APOE, LPL and IL-6 loci in young healthy subjects: Association with lipid status and obesity. BMC Res. Notes, Vol. 2., 10.1186/1756-0500-2-203

Trompeter, H.I., H. Abbad, K.M. Iwaniuk, M. Hafner and N. Renwick *et al*., 2011. MicroRNAs MiR-17, MiR-20a and MiR-106b act in concert to modulate E2F activity on cell cycle arrest during neuronal lineage differentiation of USSC. PloS One, Vol. 6 10.1371/journal.pone.0016138

Troyanskaya, O., M. Cantor, G. Sherlock, P. Brown and T. Hastie *et al*., 2001. Missing value estimation methods for DNA microarrays. Bioinformatics, 17: 520-525.

Walss-Bass, C., S.T. Weintraub, J. Hatch, J. Mintz and A.R. Chaudhuri, 2008. Clozapine causes oxidation of proteins involved in energy metabolism: A possible mechanism for antipsychotic-induced metabolic alterations. Int. J. Neuropsychopharmacol., 11: 1097-1104.

Willis, R.C. and C.W. Hogue, 2006. Searching, Viewing and Visualizing Data in the Biomolecular Interaction Network Database (BIND). In: Currrnt Protocols in Bioinformatics, Baxevanis, A.D. (Ed.). Chapter 8. John Wiley and Sons, New York, USA., pp: 1-30.

Xiao, F., Z. Zuo, G. Cai, S. Kang, X. Gao and T. Li, 2009. MiRecords: An integrated resource for microRNA-target interactions. Nucleic Acids Res., 37: D105-D110.

Yoon, P.O., M.A. Lee, H. Cha, M.H. Jeong and J. Kim *et al*., 2010. The opposing effects of CCN2 and CCN5 on the development of cardiac hypertrophy and fibrosis. J. Mol. Cell. Cardiol., 49: 294-303.

Zhang, M., L. Zeng, Y.J. Wang, Z.M. An and B.W. Ying, 2012. Associations of fibroblast growth factor 21 gene 3 untranslated region single-nucleotide polymorphisms with metabolic syndrome, obesity and diabetes in a Han Chinese population. DNA Cell Biol., 31: 547-552.