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Genetics of Metabolic Syndrome

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Summary

The metabolic syndrome (MS) is characterized by the clustering of several metabolic disorders such as increased body weight, insulin resistance, elevated plasma triglyceride levels, low HDL-cholesterol, high blood pressure and altered glucose homeostasis. Genetic and environmental factors such as low physical activity and unhealthy diet are strong determinants of the MS. Association and linkage studies on candidate genes for MS have revealed significant associations between the MS and polymorphisms in several different genes such as adiponectin, Plasma Cell Membrane Glycoprotein 1 (PC1), Hepatic Lipase, ApoA1/A5/C3/A4 Cluster, Lymphotoxin, interleukin 6 , lamin A/C, peroxisome proliferator-activated receptor-gamma 2 (PPAR γ2), angiotensinogen-1-converting enzyme (ACE), low density lipoprotein-related protein-associated protein, and Beta 2-adrenergic receptor (ADRB2) genes . In contrast, no significant associations have been reported for PPAR α, PPAR co-activator 1 α, protein tyrosine phosphatase 1 B, ACE , fatty acid binding protein 2 , tumor necrosis factor alpha (TNF-α) , insulin receptor and ADRB3 genes. Altogether, the data supports the hypothesis that complex genetic factors may be implicated in the pathogenesis of the MS.

Introduction

The metabolic syndrome (MS) is characterized by the clustering of several metabolic disorders, such as increased body weight, insulin resistance, elevated plasma triglyceride levels, low HDL-cholesterol, high blood pressure, and altered glucose homeostasis. The prevalence of the MS ranges between 15 and 25%. This prevalence increases with age, affecting 40% of subjects aged 60 years or more. Environmental factors such as low physical activity and unhealthy diet are strong determinants of the MS.

The metabolic syndrome is a disease with multifactor etiologies. Genetic factors have been found to influence the individual susceptibility to the MS. This is supported by the observation that metabolic disorders of the MS tend to clusters in families. For instance, 45% of first-degree relatives of type 2 diabetes patients are insulin resistant compared to 20% of individuals without a family history of diabetes. Patients with

type 2 diabetes also have an increased waist-to-hip ratio compared to subjects without a family history of type 2 diabetes. The heritability of obesity varies from 20 to 90% depending on whether the estimates are based on twin, adoption, or family studies. It has been reported that heritability also influences other components of the MS such as hypertension, triglyceride, and HDL-cholesterol concentrations.

There are a number of approaches available for investigating genes that confer susceptibility to a disease, including genome scanning by positional cloning and the candidate gene approach. Genome-wide linkage analyses have identified several chromosomal regions where major susceptible genes to MS may exist. Table1 shows the range of LOD scores and locations on various chromosomes where such susceptibility loci have been discovered. These loci are known to harbor several candidate genes for metabolic syndrome.

Table 1

S.No	Chromosome	LOD Signal	Location(cM)
1	2	1.0-3.4	122-180.6
2	11	1.1-3.0	131-143.1
3	16	1.1-3.2	46-78.6
4	19	1.0-3.3	80-86.4
5	22	1.0-3.4	19-20.9

Mutations and Polymorphisms in the genes

Several studies have reported significant associations between the MS and polymorphisms (SNPs) in several different genes associated with lipid metabolism, insulin resistance, adipocyte abnormality, chronic inflammation, autonomic imbalance etc. Promising candidate genes include PPAR γ2, PC1, Adiponectin, Hepatic Lipase, Apo A1, C3, A4, A5, Fatty Acid Binding Protein 2, BAR 2, RAS, Insulin Receptor substrate 1 and 2, lymphotoxin-α, interleukin 6, lamin A/C, peroxisome proliferator-activated receptor-γ (PPARγ), angiotensinogen-1-converting enzyme (ACE), low density lipoprotein-related protein-associated protein 1, β_2-adrenergic receptor (ADRB2) genes, USF1, CAPN10, HNF4a and PPARc etc.

Peroxisome Proliferator-Activated Receptors

The three peroxisome-proliferator-activated receptor (PPAR) subtypes, PPAR-gamma, PPAR-alpha and PPAR-delta, are nuclear receptors that have been the focus of extensive research during the past decade. These receptors function as lipid sensors that coordinately regulate the expression of large gene arrays and, thereby, modulate important metabolic events. Peroxisome proliferator-activated receptor (PPAR) gamma is involved mainly in adipocyte differentiation and has been suggested to play an important role in the pathogenesis of insulin resistance and atherosclerosis. The most frequently occurring PPARγ polymorphism is substitution of proline to alanine (Pro12Ala) in exon B of the PPARγ2 gene, and although many studies have been

performed on the association between this polymorphism and type 2 diabetes mellitus, insulin resistance, and obesity, the significance of such associations remains an issue of debate Many studies explored the association between the Pro12Ala polymorphism in PPAR gamma and obesity, insulin sensitivity, and type 2 diabetes (1). It appears that the Ala12 allele confers modest protection against the onset of type 2 diabetes and is also associated with an increased BMI in overweight individuals.

PPAR alpha L162V polymorphism, alone or in combination or in interaction with dietary fat intake has been also found to be associated with metabolic syndrome (2).

Plasma Cell Membrane Glycoprotein 1 (PC1)

PC1 is a class II transmembrane glycoprotein that inhibits IR tyrosine kinase activity. The A K121Q polymorphic variant in exon 4 of this PC-1 gene has been associated with insulin resistance or hyperglycemia (3, 4).

Adiponectin

Adiponectin, is an adipose-derived plasma protein and has been well established to be an important biomarker for metabolic syndrome and its complications in humans. Hypoadiponectinemia is associated with insulin resistance, type 2 diabetes, obesity, and dyslipidemia. Some of the common polymorphisms in the promoter region, exon and intron 2, and the rare nonsynonymous mutations in exon 3 of the human adiponectin gene were repeatedly shown in many studies from many different ethnic populations to associate with the phenotypes related to body weight, glucose metabolism, insulin sensitivity, and risk of type 2 diabetes mellitus and coronary artery disease. The SNP276 may affect impaired glucose tolerance and hypoadiponectinemia (5).

The association of adiponectin genetic variations with dyslipidemia and blood pressure have been less explored. The common polymorphisms and rare mutations of the human adiponectin gene itself were demonstrated to associate with differential expression of adiponectin at the plasma protein level and mRNA level in adipose tissue. The PPARgamma2 Pro12Ala variants were also shown to influence insulin sensitivity in interaction with adiponectin genotype or to influence plasma adiponectin levels. However, the results were not consistent. Three genome-wide scans for the loci that regulate plasma adiponectin concentration suggest that further exploration on chromosomes 5, 9, 14, 15, and 18 is required. These human genetic studies on adiponectin and the metabolic syndrome strongly suggest that adiponectin is one of the causative factors in its pathogenesis and provide significant insights into the genetic makeup of the metabolic syndrome

Hepatic Lipase (HL)

High HL activity is associated with reduced HDL2 cholesterol levels and is

affected by dietary fat intake and selected medications. There is evidence for an interaction of the HL promoter polymorphism with visceral obesity and dietary fat intake. Several polymorphisms, G 250 A , C 514 T, T 710 C, A 763 G are known to be associated with HL activity (6).

A recent linkage and association analysis revealed variation at the APOA1/C3/A4/A5 gene cluster contributes to Familial hypercholesterolemia transmission in a substantial proportion of Northern European families. Apo CIII: TG-rich lipoproteins are often increased in metabolic syndrome. The T-455C polymorphism of Apo CIII are shown to be associated with increased Apo CIII and TG levels. Apo CIII-455C was found to increases risk of CAD in MS. Obesity was less frequent in MS carriers of the -455C allele than in MS non carriers (21.6% vs. 34.8%, $P < 0.05$)

APOA V

Several polymorphisms of Apo V (-1131C (originally referred to as SNP3), Ser19Trp , have been shown to be have associations with TG in healthy and non-smoking subjects. T-1131C was found to be associated with higher concentrations of plasma TG

Fatty Acid Binding Protein 2(FABP 2) and Beta Adrenergic Receptor 2 (BAR 2)

Both these proteins link the inflammatory and lipid-mediated pathways and have been are implicated in Atherosclerosis, Obesity, Insulin resistance, Type 2 diabetes and Fatty liver disease . Ala54Thr polymorphism of FABP2 and Trp64Arg polymorphism of BAR 2 has been implicated in MS (7).

Renin Angiotensin System (RAS)

Helps maintain blood pressure and salt homeostasis. Polymorphisms of RAS genes, namely ACE insertion/deletion (I/D), Angiotensinogen (AGT). M235T, Angiotensin II type 1 receptor (AT1R). A1166C polymorphisms have been studied for their association with various cardiovascular disorders, including metabolic syndrome. D-allele and DD genotype of ACE I/D has been found to be associated with development of obesity, insulin resistance, type 2 diabetes, dyslipoproteinemias and ischemic heart disease and myocardial infarction in some of the studies whereas many studies have failed to show any such association (8).

There has been some evidence to support an association between the AGT polymorphism and insulin resistance (9).

Angiotensin II type I receptor (AGTR1) A1166C appears to predispose to favourable anthropometric and metabolic traits, relative to cardiovascular risk (10).

USF1

USF1 is a transcription factor of the c-myc family [11] that is known to regulate several genes involved in glucose and lipid metabolism, including apolipoproteins CIII, AII and E, hormone sensitive lipase, fatty acid synthase, glucokinase, the

glucagon receptor, ATP binding cassette A1 and rennin (12). The USF1 gene is located on human chromosome 1q22–q23 (13). This locus on chromosome 1 has been linked to familial combined hyperlipidaemia (FCHL), a condition characterized by elevated total cholesterol and / or triglycerides, in Finnish families (14) and is also located close to type 2 diabetes linkage peaks in other populations (15, 16). Subsequently, the USF1 gene has been linked and associated with FCHL, and haplotypes of USF1 explain the linkage peak associated with disease, triglycerides, cholesterol, small dense low-density lipoprotein and apolipoprotein B. Furthermore, target genes for this transcription factor include many other genes associated with hypertension and diabetes. Subsequent candidate gene studies will determine if there is an association between components of metabolic syndrome and SNPs of the USF1 gene.

CAPN10

Calpains are ubiquitously expressed cysteine proteases that regulate a variety of cellular functions. CAPN10 is expressed in B cells where evidence suggests it mediates apoptosis and insulin exocytosis (17, 18), in fat and muscle CAPN10 modifies insulin-mediated glucose transport (18). It also appears to be involved in myoblast / pre-adipocyte differentiation (19, 20). CAPN10 was the first type 2 diabetes gene identified by a genome-wide scan of Mexican–American families. Initially, linkage was found on chromosome 2 (LOD 4.03) (21) and the gene was identified as calpain 10. Three intron variants account for most of the haplotype diversity and for 14% of the population-attributable risk of type 2 diabetes in Mexican–Americans (22). Meta-analyses of association studies assessing CAPN10 and type 2 diabetes risk have confirmed a role for CAPN10 polymorphisms in type 2 diabetes susceptibility (23), increasing risk by _30% for type 2 diabetes (24). In one of the analyses, SNP44, a rare CAPN10 allele, was shown to be over-transmitted from heterozygous parents to their affected offspring with type 2 diabetes. In Pima Indians, a CAPN10 polymorphism (SNP43) correlates with impaired insulin action and reduced expression of CAPN10 in the skeletal muscle of prediabetic subjects (25). CAPN10 polymorphisms have also been associated with insulin secretion, adipocyte biology and microvascular function. This suggests that CAPN10 plays a role in the metabolic syndrome.

HNF4a

HNF4a is a hepatic nuclear factor that controls expression of many essential genes in the liver, gut, kidney and B cells, and plays an important role in maintaining glucose homeostasis (26). It is also involved in B-cell development, and mutations of HNF4a are a rare cause of MODY (27). The gene for HNF4a has been mapped to chromosome 20. Chromosome 20 has shown evidence of linkage to type 2 diabetes (LOD score 2.48) (28), and an

association between a common HNF4a polymorphism in the upstream promoter region of the gene has been found in an Ashekenazi Jewish population. Furthermore, a case control association study searching for diabetes susceptibility variants at 20q13 found 10 associated polymorphisms located in the promoter regions and exons 1–3 of the HNF4a gene (28). These data suggest variants located near or within the HNF4 gene increase susceptibility to type 2 diabetes.

PPARc

Variants of the PPARc gene are strong candidates for conferring susceptibility to type 2 diabetes and obesity, because PPARc regulates adipocyte differentiation, and lipid and glucose metabolism (29). Two PPARc isoforms, PPARc1 and PPARc2, have been characterized, and are encoded by a single PPAR gamma gene. The PPARc gene has been mapped to human chromosome 3p25 (30). One of the first examples of a meta-analysis in complex disease demonstrated that the Pro12Ala variant of PPARc2 is associated with predisposition to type 2 diabetes (31). Evidence suggests a gene–nutrient interaction at the PPARc2 locus in nondiabetic subjects(32). BMI was shown to vary with the ratio of dietary polyunsaturated to saturated fat (P:S) – in Ala12 carriers, BMI was low when the P:S ratio was high and was elevated when the P:S ratio was low. This gene–nutrient interaction suggests that effects of the Pro12Ala polymorphism could depend on the most common diets in the populations studied and may explain the conflicting results of previous studies. Furthermore, in a single study, the Finnish diabetes prevention study, the Ala12 allele was associated with increased risk of progression to type 2 diabetes. However, the Ala12Ala genotype was found to be associated with increased weight loss in response to lifestyle intervention and less progression from IGT to diabetes (33). Thus, lifestyle intervention can reverse the diabetogenic risk of the Ala allele.

In contrast, no significant associations have been reported for PPARα [2], PPAR co-activator 1α (34), protein tyrosine phosphatase 1 B (35), ACE (36), fatty acid binding protein 2 (37) tumor necrosis factor-α (TNF-α) (38) , insulin receptor (39) and ADRB3 (40) and (41) genes. Altogether, these data support the hypothesis that complex genetic factors may be implicated in the pathogenesis of the MS.

UCP3

UCP3 is a mitochondrial membrane transporter mainly expressed in skeletal muscle. This protein uncouples oxidative ATP phosphorylation and if dysregulated, might lead to thermogenesis and energy balance disorders. The UCP3 -55 C/T SNP is associated with higher UCP3 mRNA expression levels (42) and fat mass (43-47). The relation between this SNP and type 2 diabetes is controversial, with a lower risk of type 2 diabetes in this population sample (48, 27) and a higher risk in Chinese (49). Despite the major contribution of increased body mass in the clustering of metabolic disorders and the persistent association

between the UCP3 -55 C/T SNP and increased fat mass, there was no significant association between this SNP and the MS

Leptin

It is an adipocyte-secreted hormone acting in the brain to control energy homeostasis. Its main effect is to regulate appetite and energy balance (50). Pathogenic mutations in the leptin (LEP) gene leading to the absence of any functional hormone are associated with early-onset morbid obesity (51, 52). The LEP 5'UTR +19 G/A SNP is associated with lower leptin concentrations in obese individuals (53). In the present population sample, this SNP was not significantly associated with the MS.

G proteins mediate many pathways including the β-adrenergic signalling pathways. A polymorphism in the β_2-adrenergic receptor is associated with the MS (54) , suggesting a possible implication of the adrenergic pathway in the clustering of MS. The GNB3 C825T SNP is functional and has been associated with several phenotypes such as dyslipidemia, hypertension, obesity, and diabetes mellitus (55, 56). Furthermore, a study including 806 Japanese subjects (80% men) showed the CC genotype was protective against the onset of the clustering of obesity, hypertriglyceridemia, hypertension, and diabetes mellitus (56).

Given the fact that the prevalence of coronary heart disease stroke and all-cause mortality is increased threefold in subjects with the MS (57), even in the absence of baseline CVD and diabetes (58), it is important to better understand the factors that increase the susceptibility to the development of the MS. Genetic association studies may help in this respect. We could not reveal major associations between any of the studied polymorphisms in FATP1, UCP3, TNF-α, LEP, and GNB3 genes and the MS, suggesting that these polymorphisms are not major risk factors for the MS.

As is evident, association of gene variants, insulin resistance and dyslipidaemia is complex and has resulted in inconsistent findings in different studies. Moreover, the genetic studies till date generally have been of a small size and cross sectional in approach which limits analysis of trends in insulin sensitivity and lipid and lipoprotein levels in association with different gene variants. Ethnic differences in prevalence of various alleles have further led to a large variability in the results. However, even taking these limitations into account, gene variants and their interaction with environment appear to modulate glucose and lipoprotein metabolism.

Given the fact that the prevalence of coronary heart disease, stroke and all-cause mortality is increased several folds in subjects with the MS, even in the absence of baseline cardiovascular diseases and diabetes, it is important to better understand the factors that increase the susceptibility to the development of the MS. Genetic association studies may help in this respect

References

1. Meirhaeghe A, Amouyel P (2004). Impact of genetic variation of PPARgamma in humans. *Mol Genet Metab* **83**(1-2): 93-102.
2. Robitaille J, Brouillette C, Houde A, Lemieux S, Perusse L, Tchernof A, Gaudet D, Vohl MC (2004). Association between the PPARalpha-L162V polymorphism and components of the metabolic syndrome. *J Hum Genet* **49**(9): 482-489.
3. Gu HF, Almgren P, Lindholm E, Frittitta L, Pizzuti A, Trischitta V, Groop LC (2000). Association between the human glycoprotein PC-1 gene and elevated glucose and insulin levels in a paired-sibling analysis. *Diabetes* **49**(9): 1601-1603.
4. Pizzuti A, Frittitta L, Argiolas A, Baratta R, Goldfine ID, Bozzali M, Ercolino T, Scarlato G, Iacoviello L, Vigneri R *et al* (1999). A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. *Diabetes* **48**(9): 1881-1884.
5. Gonzalez-Sanchez JL, Zabena CA, Martinez-Larrad MT, Fernandez-Perez C, Perez-Barba M, Laakso M, Serrano-Rios M (2005). An SNP in the adiponectin gene is associated with decreased serum adiponectin levels and risk for impaired glucose tolerance. *Obes Res* **13**(5): 807-812.
6. Deeb SS, Zambon A, Carr MC, Ayyobi AF, Brunzell JD (2003). Hepatic lipase and dyslipidemia: interactions among genetic variants, obesity, gender, and diet. *J Lipid Res* **44**(7): 1279-1286.
7. Ishii T, Hirose H, Kawai T, Hayashi K, Maruyama H, Saito I, Saruta T (2001). Effects of intestinal fatty acid-binding protein gene Ala54Thr polymorphism and beta3-adrenergic receptor gene Trp64Arg polymorphism on insulin resistance and fasting plasma glucose in young to older Japanese men. *Metabolism* **50**(11): 1301-1307.
8. Lee YJ, Tsai JC (2002). ACE gene insertion/deletion polymorphism associated with 1998 World Health Organization definition of metabolic syndrome in Chinese type 2 diabetic patients. *Diabetes Care* **25**(6): 1002-1008.
9. Sheu WH, Lee WJ, Jeng CY, Young MS, Ding YA, Chen YT (1998). Angiotensinogen gene polymorphism is associated with insulin resistance in nondiabetic men with or without coronary heart disease. *Am Heart J* **136**(1): 125-131.
10. Abdollahi MR, Gaunt TR, Syddall HE, Cooper C, Phillips DI, Ye S, Day IN (2005). Angiotensin II type I receptor gene polymorphism: anthropometric and metabolic syndrome traits. *J Med Genet* **42**(5): 396-401.
11. Gregor PD, Sawadogo M, Roeder RG (1990). The adenovirus major late transcription factor USF is a member of the helix-loop-helix group of regulatory proteins and binds to DNA as a dimer. *Genes Dev* **4**(10): 1730-1740.
12. Pajukanta P, Lilja HE, Sinsheimer JS, Cantor RM, Lusis AJ, Gentile M, Duan XJ, Soro-Paavonen A, Naukkarinen J, Saarela J *et al* (2004). Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). *Nat Genet* **36**(4): 371-376.
13. Shieh BH, Sparkes RS, Gaynor RB, Lusis AJ (1993). Localization of the gene-encoding upstream stimulatory factor (USF) to human chromosome 1q22-q23. *Genomics* **16**(1): 266-268.

14. Pajukanta P, Nuotio I, Terwilliger JD, Porkka KV, Ylitalo K, Pihlajamaki J, Suomalainen AJ, Syvanen AC, Lehtimaki T, Viikari JS *et al* (1998). Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. *Nat Genet* **18**(4): 369-373.

15. Xiang K, Wang Y, Zheng T, Jia W, Li J, Chen L, Shen K, Wu S, Lin X, Zhang G *et al* (2004). Genome-wide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese: significant linkage to chromosome 6q21-q23 and chromosome 1q21-q24. *Diabetes* **53**(1): 228-234.

16. Das SK, Hasstedt SJ, Zhang Z, Elbein SC (2004). Linkage and association mapping of a chromosome 1q21-q24 type 2 diabetes susceptibility locus in northern European Caucasians. *Diabetes* **53**(2): 492-499.

17. Johnson JD, Han Z, Otani K, Ye H, Zhang Y, Wu H, Horikawa Y, Misler S, Bell GI, Polonsky KS (2004). RyR2 and calpain-10 delineate a novel apoptosis pathway in pancreatic islets. *J Biol Chem* **279**(23): 24794-24802.

18. Sreenan SK, Zhou YP, Otani K, Hansen PA, Currie KP, Pan CY, Lee JP, Ostrėga DM, Pugh W, Horikawa Y *et al* (2001). Calpains play a role in insulin secretion and action. *Diabetes* **50**(9): 2013-2020.

19. Patel YM, Lane MD (1999). Role of calpain in adipocyte differentiation. *Proc Natl Acad Sci U S A* **96**(4): 1279-1284.

20. Yajima Y, Kawashima S (2002). Calpain function in the differentiation of mesenchymal stem cells. *Biol Chem* **383**(5): 757-764.

21. Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, Wapelhorst B, Spielman RS, Gogolin-Ewens KJ *et al* (1996). A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nat Genet* **13**(2): 161-166.

22. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE *et al* (2000). Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* **26**(2): 163-175.

23. Weedon MN, Schwarz PE, Horikawa Y, Iwasaki N, Illig T, Holle R, Rathmann W, Selisko T, Schulze J, Owen KR *et al* (2003). Meta-analysis and a large association study confirm a role for calpain-10 variation in type 2 diabetes susceptibility. *Am J Hum Genet* **73**(5): 1208-1212.

24. Song Y, Niu T, Manson JE, Kwiatkowski DJ, Liu S (2004). Are variants in the CAPN10 gene related to risk of type 2 diabetes? A quantitative assessment of population and family-based association studies. *Am J Hum Genet* **74**(2): 208-222.

25. Baier LJ, Permana PA, Yang X, Pratley RE, Hanson RL, Shen GQ, Mott D, Knowler WC, Cox NJ, Horikawa Y *et al* (2000). A calpain-10 gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. *J Clin Invest* **106**(7): R69-73.

26. Stoffel M, Duncan SA (1997). The maturity-onset diabetes of the young (MODY1) transcription factor HNF4alpha regulates expression of genes required for glucose transport and metabolism. *Proc Natl Acad Sci U S A* **94**(24): 13209-13214.

27. Sander M, German MS (1997). The beta cell transcription factors and development of the pancreas. *J Mol Med* **75**(5): 327-340.

28. Silander K, Mohlke KL, Scott LJ, Peck EC, Hollstein P, Skol AD, Jackson AU, Deloukas P, Hunt S, Stavrides G *et al* (2004). Genetic variation near the hepatocyte nuclear factor-4 alpha gene predicts susceptibility to type 2 diabetes. *Diabetes* **53**(4): 1141-1149.

29. Auwerx J (1999). PPARgamma, the ultimate thrifty gene. *Diabetologia* **42**(9): 1033-1049.

30. Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, Hsieh L, Greene G, Nimer SD (1995). Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. *Gene Expr* **4**(4-5): 281-299.

31. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C *et al* (2000). The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* **26**(1): 76-80.

32. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ (2001). Evidence for gene-nutrient interaction at the PPARgamma locus. *Diabetes* **50**(3): 686-689.

33. Lindi VI, Uusitupa MI, Lindstrom J, Louheranta A, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M *et al* (2002). Association of the Pro12Ala polymorphism in the PPAR-gamma2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. *Diabetes* **51**(8): 2581-2586.

34. Ambye L, Rasmussen S, Fenger M, Jorgensen T, Borch-Johnsen K, Madsbad S, Urhammer SA (2005). Studies of the Gly482Ser polymorphism of the peroxisome proliferator-activated receptor gamma coactivator 1alpha (PGC-1alpha) gene in Danish subjects with the metabolic syndrome. *Diabetes Res Clin Pract* **67**(2): 175-179.

35. Dahlman I, Wahrenberg H, Persson L, Arner P (2004). No association of reported functional protein tyrosine phosphatase 1B 3' UTR gene polymorphism with features of the metabolic syndrome in a Swedish population. *J Intern Med* **255**(6): 694-695.

36. Costa LA, Canani LH, Maia AL, Gross JL (2002). The ACE insertion/deletion polymorphism is not associated with the metabolic syndrome (WHO Definition) in Brazilian type 2 diabetic patients. *Diabetes Care* **25**(12): 2365-2366; author reply 2366-2367.

37. Erkkila AT, Lindi V, Lehto S, Pyorala K, Laakso M, Uusitupa MI (2002). Variation in the fatty acid binding protein 2 gene is not associated with markers of metabolic syndrome in patients with coronary heart disease. *Nutr Metab Cardiovasc Dis* **12**(2): 53-59.

38. Lee SC, Pu YB, Thomas GN, Lee ZS, Tomlinson B, Cockram CS, Critchley JA, Chan JC (2000). Tumor necrosis factor alpha gene G-308A polymorphism in the metabolic syndrome. *Metabolism* **49**(8): 1021-1024.

39. Thomas GN, Tomlinson B, Chan JC, Lee ZS, Cockran CS, Critchley JA (2000). An insulin receptor gene polymorphism is associated with diastolic blood pressure in Chinese subjects with components of the metabolic syndrome. *Am J Hypertens* **13**(7): 745-752.

40. Thomas GN, Tomlinson B, Chan JC, Young RP, Critchley JA (2000). The Trp64Arg polymorphism of the beta3-adrenergic receptor gene and obesity in Chinese subjects with components of the metabolic syndrome. *Int J Obes Relat Metab Disord* **24**(5): 545-551.

41. Rissanen J, Kuopusjarvi J, Pihlajamaki J, Sipilainen R, Heikkinen S, Vanhala M, Kekalainen P, Kuusisto J, Laakso M (1997). The Trp64Arg polymorphism of the beta 3-Adrenergic receptor gene. Lack of association with NIDDM and features of insulin resistance syndrome. *Diabetes Care* **20**(8): 1319-1323.

42. Schrauwen P, Xia J, Walder K, Snitker S, Ravussin E (1999). A novel polymorphism in the proximal UCP3 promoter region: effect on skeletal muscle UCP3 mRNA expression and obesity in male non-diabetic Pima Indians. *Int J Obes Relat Metab Disord* **23**(12): 1242-1245.

43. Cassell PG, Saker PJ, Huxtable SJ, Kousta E, Jackson AE, Hattersley AT, Frayling TM, Walker M, Kopelman PG, Ramachandran A *et al* (2000). Evidence that single nucleotide polymorphism in the uncoupling protein 3 (UCP3) gene influences fat distribution in women of European and Asian origin. *Diabetologia* **43**(12): 1558-1564.

44. Damcott CM, Feingold E, Moffett SP, Barmada MM, Marshall JA, Hamman RF, Ferrell RE (2004). Genetic variation in uncoupling protein 3 is associated with dietary intake and body composition in females. *Metabolism* **53**(4): 458-464.

45. Halsall DJ, Luan J, Saker P, Huxtable S, Farooqi IS, Keogh J, Wareham NJ, O'Rahilly S (2001). Uncoupling protein 3 genetic variants in human obesity: the c-55t promoter polymorphism is negatively correlated with body mass index in a UK Caucasian population. *Int J Obes Relat Metab Disord* **25**(4): 472-477.

46. Lindholm E, Klannemark M, Agardh E, Groop L, Agardh CD (2004). Putative role of polymorphisms in UCP1-3 genes for diabetic nephropathy. *J Diabetes Complications* **18**(2): 103-107.

47. Otabe S, Clement K, Dina C, Pelloux V, Guy-Grand B, Froguel P, Vasseur F (2000). A genetic variation in the 5' flanking region of the UCP3 gene is associated with body mass index in humans in interaction with physical activity. *Diabetologia* **43**(2): 245-249.

48. Meirhaeghe A, Amouyel P, Helbecque N, Cottel D, Otabe S, Froguel P, Vasseur F (2000). An uncoupling protein 3 gene polymorphism associated with a lower risk of developing Type II diabetes and with atherogenic lipid profile in a French cohort. *Diabetologia* **43**(11): 1424-1428.

49. Shen H, Xiang K, Jia W (2002). [Effects of uncoupling protein 3 gene -55 C—>T variant on lipid metabolism, body fat, its distribution and non-insulin-dependent diabetes mellitus in Chinese]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* **19**(4): 317-321.

50. Meier U, Gressner AM (2004). Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin Chem* **50**(9): 1511-1525.

51. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA *et al* (1997). Congenital leptin deficiency is

associated with severe early-onset obesity in humans. *Nature* **387**(6636): 903-908.

52. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD (1998). A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Genet* **18**(3): 213-215.

53. Hager J, Clement K, Francke S, Dina C, Raison J, Lahlou N, Rich N, Pelloux V, Basdevant A, Guy-Grand B *et al* (1998). A polymorphism in the 5' untranslated region of the human ob gene is associated with low leptin levels. *Int J Obes Relat Metab Disord* **22**(3): 200-205.

54. Dallongeville J, Helbecque N, Cottel D, Amouyel P, Meirhaeghe A (2003). The Gly16—>Arg16 and Gln27—>Glu27 polymorphisms of beta2-adrenergic receptor are associated with metabolic syndrome in men. *J Clin Endocrinol Metab* **88**(10): 4862-4866.

55. Siffert W (2005). G protein polymorphisms in hypertension, atherosclerosis, and diabetes. *Annu Rev Med* **56**: 17-28.

56. Yamamoto M, Abe M, Jin JJ, Wu Z, Tabara Y, Mogi M, Kohara K, Miki T, Nakura J (2004). Association of GNB3 gene with pulse pressure and clustering of risk factors for cardiovascular disease in Japanese. *Biochem Biophys Res Commun* **316**(3): 744-748.

57. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L (2001). Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* **24**(4): 683-689.

58. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT (2002). The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *Jama* **288**(21): 2709-2716.