

## Genetic aspects of obesity

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### Abstract

The paper reviews recent problems in understanding of the genetic basis and gene/gene, as well as gene/environment interaction in the development of obesity and its complications.

### Introduction

In recent years, obesity has become a major public health problem owing to its prevalence, which stands at more than 25% in certain countries, with its alarming increase in children. The molecular mechanisms responsible for fat mass accumulation and maintenance are still to be elucidated. Obesity results from the interaction of environmental factors ( high calorie density diet and reduction in physical activity) and hereditary factors. This has been shown by numerous epidemiological studies carried out in large and different populations which vary ethnically (twins brought up together or separately, adopted children, nuclear families, etc.) [1-5].

Obesity has a very heterogeneous phenotypic expression and the molecular mechanisms involved in its development are diverse. According to several studies, 30 to 80% of weight variation might be attributed to genetic factors [1-5]. Today, the participation of genetic factors in the development of obesity can be summarized as follows:

- single mutations contribute to the development of obesity (monogenic obesity). These forms of obesity are rare, but very severe and generally start in childhood [6];
- several genetic variants interact with an "at-risk" environment what results in the development of common obesity (polygenic obesity).

Genetic variation of each susceptibility gene, taken individually, has a minimal effect on weight gain. However, the cumulative contribution of these genes becomes significant when there is an interaction with environmental factors predisposing to their phenotypic expression (overeating, reduction in physical activity, hormonal changes, or socioeconomic factors). The common disease/common variant hypothesis for obesity has been proposed

[7]. It suggests that the genetic risk for obesity is due to the coexistence of disease-promoting frequent alleles in the organism. As a consequence, the percentage of developed obesity attributed to them is high (attributable risk). This is a current hypothesis in understanding of "multifactorial diseases". However, even strong believers of this hypothesis agree, that in certain circumstances, the role of rare variant alleles may intervene (8,9) . The risk for common obesity could be also attributed to the presence of the certain number of loci, each with the multifactorial disease - obesity predisposing, low frequency alleles [8]. At present time, these two hypotheses are valid, because the genetic approach has not allowed one to be confirmed more than the other [9].

Numerous studies using both candidate gene and genome-wide approaches have been used to identify genes predisposing to obesity. As a result, the Human Obesity Gene Map currently updated was published in March 2005. It was the 11th from the series of reviews collecting and reporting the publications about genes related to obesity [10]. In the current work we present the problems with linkage of gene polymorphism and phenotype expressed by metabolic traits.

### Monogenic forms of obesity

Obesity is seldom caused by a single gene defect in general population [11]. Table 1 shows the gene variants identified so far in families with obesity, or the variants which were only found in obese individuals [10].

**Table 1** Genes causing monogenic forms of obesity in humans.

Gene	Reference
Leptin (LEP)	[12, 13; 14]
Leptin receptor (LEPR)	[15]
Pro-opiomelanocortin (POMC)	[16; 17]
Prohormone convertase-1 (PC1) = Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1)	[18, 19]
Melanin-concentrating hormone receptor 1 (MCHR1) = G protein-coupled receptor 24 (GPR24)	[20]
Melanocortin-3 receptor (MC3R)	[21]
Melacortin-4 receptor (MC4R)	[10, 22, 23]
Corticotropin-releasing hormone receptor-1	[24]

(CRHR1)	
Corticotropin-releasing hormone receptor -2 (CRHR2)	[24]
bHLH-PAS transcription factor (SIM1)	[25, 26]

The cloning of the ob gene in the mouse and its human homologue, Leptin [27] provided the first example of a causal relationship between a mutation and obesity. Two different mutations disrupting the structure of the Leptin gene have so far been identified in 6 morbidly obese children [12,13,14]. Treatment of these children with recombinant leptin protein, dramatically normalised weight, puberty as well as reduced the most of metabolic syndrome symptoms [28]. It is however not the case of the common obesity, since obese individuals demonstrate the elevated serum leptin levels and leptin resistance [29,30]. For the rare forms of obesity, mutations of the melanocortin receptor (MC4R) were identified, and their prevalence has been estimated to be as high as 2-4% among obese children [10, 34,35,]. The degree of obesity in individuals carrying the MC4R mutation varies and these individuals are usually also taller. A recent meta-analysis suggests that the common allele of the Val103Ile variant in the coding region of the MC4R is associated with obesity, whereas the rare allele (Ile103) (with a frequency of 4%) is more common in lean individuals [36]. The Ile103 allele has been found to be also associated with lower BMI [37].

### **Obesity linked to the large-scale chromosomal mutations**

In addition to genetic defects mostly affecting body weight, numerous syndromes featuring obesity as one of the symptoms have been mapped to certain chromosomal loci, and for some of these cases the underlying gene has been identified [10]. Prader-Willi is the most common syndrome affecting every 16,000-25,000 newborns a year [31,32]. The Prader-Willi syndrome is an imprinting disorder that is usually caused by a deletion of paternally inherited chromosome 15q region.

The origin of obesity is more complex in Bardet-Biedl syndrome (BBS), which is characterized by six main features such as: rod-cone-dystrophy (the most frequent phenotype), polydactyly, learning disabilities, hypogonadism in males, renal abnormalities and obesity. In BBS patients, obesity occurs with an early onset, usually arising within the first few years of life. The genetic basis of BBS is typically autosomal recessive, however, the occurrence of triallelic inheritance has been suggested in some families [77].

The genetic background of the common forms of obesity

The first evidence that genetics is important in common, non-syndromic obesity came from a study that was published nearly 30 years ago. In 1977, the National Heart, Lung and Blood Institute (NHLBI) Twin Study first indicated the possibility that the observed familial aggregation for obesity was due to genetic factors rather than environment [78].

Subsequently, in 1986, Stunkard used 1,974 monozygotic and 2,097 dizygotic twin pairs, and estimated a heritability value for weight of 0.78, which increased to 0.81 after the 25-year follow-up [2]. These values were similar to the heritability value of 0.80 for height that was estimated in the same study.

A children adoption study showed at the same time similar results in support of a genetic

influence on body weight, with adopted children having body sizes more similar to those of their biological parents than their adopted parents across the whole range of body size [3]. These studies were effectively combined in a seminal paper in 1990 that examined identical and fraternal twins that were reared together and apart [4]. Similarly to the previous studies the intra-pair correlation coefficients for obesity phenotypes of 0.70 for men and 0.66 for women were reported. Shared environment seemed to have no measurable effect and non-shared personal environment contributed about 30% of the variance (2,4).

Multiple genome-wide scans have been performed for obesity and traits related to body composition [10]. Typically in complex disorders, first identification of a region linked to the disease does not automatically lead to a replication in a follow-up study performed in another study sample. However, evidence of linkage (lod score > 3.0 or p < 0.001) for obesity and traits related to body composition has been identified in several studies for certain chromosome regions (10).

More than three hundred gene polymorphisms have been found to be associated with common forms of obesity, although much smaller number of them has been confirmed by the studies carried out in different ethnic populations[10]. This can be explained by the several causes including the small risk that the disease associated variant presents, small study sample size, and the linkage disequilibrium between the actual causative variants and the variants tested in the study. The gene-gene or gene environment interactions and phenotypic heterogeneity may also complicate the analysis, if the study populations have the different haplotype backgrounds or different environmental exposures. Despite these difficulties, the initial associations of some genes with obesity or related phenotypes have been replicated (reviewed in 10). The results of many genetic studies, that concern a large number of genes and chromosomal regions, are reported each year in the international journal: Obesity Research [10]. We will not give details on all studies, but will provide illustrative examples. Table 2 illustrates the pathways in which genetic polymorphisms may affect the physiological pathways involved in the regulation of energy balance, hereby increasing the susceptibility to developing obesity in a given environmental setting. Examples of putative candidate genes are given for each pathway.

**Table 2.** Different mechanisms by which genetics is expected to play a role in the development of obesity; examples of putative candidate genes are given for each category. The genes are annotated with the approved gene symbol (Human Genome Nomenclature Database). Adapted from reference 9 and 10

<b>Metabolic pathway</b>	<b>Physiological mechanism</b>	<b>Candidate genes/proteins</b>
<i>Adipose tissue</i>		
Development and accumulation	Adipocyte differentiation, fat accumulation	<i>FOXC2, PPARA, PPARD, PPARG, RXRA, RXRB</i>
Metabolic function	Balance between lipids release and accumulation	<i>VLDLR, LIPE, LPL, SCD, UCP2, ADRB1, ADRB2, ADRB3, ADRA2A, ADRA2B, INSR, FABP; CD36</i>

Endocrine function	Signals from adipose tissue to central regulation of energy balance	<i>LEP, LEPR, NPPA, SPARC, TNF, IL6, AMP1,</i>
<i>Energy intake</i>		
Central	Hypothalamic neurotransmitters or receptors	<i>NPY, NPYR, POMC, MC4R, LEPR, CART, 5HT2C, CCKAR, AGRP</i>
Peripheral	Hormones or other signaling compounds involved in appetite regulation	<i>CCK, APOA-IV, GHRL, PPY, GLP GIP</i>
Food preferences	Preference for sweet, fat, aversion to certain fruits and vegetables due to high sensitivity to bitter taste.	<i>TAS1R, TAS2R</i>
<i>Energy expenditure</i>		
Central	Hypothalamic neurotransmitters or receptors	<i>MC4R, Dopamin 2R, NPYR</i>
Mediator	Sympatho-adrenergic system	<i>ADRB1, ADREB2, ADRB3, ADRA2A, ADRA2B</i>
Effectors	EE as such, fat oxidation	<i>UCP1, UCP2, UCP3</i>

**Abbreviations:**

*ADRB1*- beta-1 adrenergic receptor; *ADRB2*-beta-2 adrenergic receptor; *ADRA2A* -alpha-2A-, receptor *ADRA2B* -alpha-2B-, receptor; *ADRB3*-beta-3 adrenergic receptor; adrenergic;; *AGRP*- agouti-related protein; *AMP1*- carcinoembryonic antigen-related cell adhesion molecule pseudogene 1; *APOA-IV*- apolipoprotein A-IV; *CCK*- cholecystokinin; *CCKAR*- cholecystokinin A receptor; *Dopamin 2R*- dopaminergic D2 receptor; *FABP*: - Fatty acid binding protein; *FOXC2*- forkhead box protein C2; *GHRL*- ghrelin; *GLP*- glucagon-like peptide; *5HT2C*-5-hydroxytryptamine 2C receptor; *IL6*- interleukin 6 (interferon, beta 2); *INSR*- insulin receptor; *LEP*-leptin; *LEPR*- leptin receptor; *LIPE*- lipase, hormone-sensitive; *LPL*- lipoprotein lipase; *MC4R*- melanocortin 4 receptor; *NPPA*- natriuretic peptide precursor A; *NPY*- neuropeptide Y; *NPYR*- neuropeptide Y receptor; *POMC*- proopiomelanocortin; *PPARA*- peroxisome proliferative activated receptor, alpha; *PPARD*- peroxisome proliferative activated receptor, delta; *PPARG*- peroxisome

proliferative activated receptor, gamma; *PPY*- pancreatic polypeptide; *RXRA*- retinoic acid receptor RXR-alpha; *RXRB*- retinoic acid receptor RXR-beta; *SCD*- stearyl-CoA desaturase (delta-9-desaturase); *SPARC*- secreted protein, acidic, cysteine-rich (osteonectin); *TAS1R*- taste receptor, type 1; *TAS2R*- taste receptor, type 2; *TNF*- tumor necrosis factor; *UCP1*- uncoupling protein 1 (mitochondrial, proton carrier); *UCP2*- uncoupling protein 2 (mitochondrial, proton carrier); *UCP3*- uncoupling protein 3 (mitochondrial, proton carrier); *VLDLR*- very low-density lipoprotein receptor;

The genes for which at least five different studies found association with common obesity or obesity related phenotypes include Adiponectin; Adrenergic: beta-2- and beta-3- receptors (ADRB2 and ADRB3); Guanine nucleotide binding protein (G protein), beta polypeptide 3 (GNB3); Interleukin 6 (interferon, beta 2) (IL6); Insulin; Leptin (LEP), Leptin receptor (LEPR); Lamin A/C (LIPE); Nuclear receptor subfamily 3, group C member 1 (NR3C1); PPARG; Tumor necrosis factor TNF superfamily, member 2 (TNF); as well as Uncoupling proteins 1, 2 and 3 (mitochondrial, proton carrier) (UCP1, UCP2 and UCP3) [reviewed in 10]. Recently marked progress has been made in the identification of obesity predisposing genes using genome-wide linkage and subsequent fine mapping studies [11,38, 39,]. The benefit of the positional cloning strategy is that it does not rely on any pre-existing knowledge of the genes that underlie the investigated trait. Particularly, for conditions such as obesity this may be useful since there is as yet limited information available, for instance regarding the appetite regulation. The first candidate gene for obesity identified through the genome wide approach was Glutamate decarboxylase 1 (GAD2) on chromosome 10p12 [38]. It encodes the glutamic acid decarboxylase enzyme GAD65 which is suggested to be connected with obesity by the hypothalamic regulation of food intake by the formation of the  $\gamma$ -aminobutyric acid (GABA) from the glutamic acid. GABA functions together with neuropeptide Y in the paraventricular nucleus increasing food intake. GAD2 polymorphisms were demonstrated to be associated with childhood obesity, high birth weight and binge eating [40]. However, the recent replication study on German, American and Canadian populations did not confirm the significant association of this gene with obesity [41]. Another interesting example is the Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene in chromosome 6q22-23. This 6q22-23 region has been previously demonstrated to be linked to obesity [42, 43], BMI [42], insulin secretion [44, 45] and T2DM [46, 47, 48, 49]. Recently, variants in ENPP1 were identified to be connected with obesity and T2DM [40]. ENPP1 encodes a prohormone convertase-1, the enzyme which inhibits the insulin receptor kinase activity and subsequent cellular signaling of insulin [50, 51]. ENPP1 is also involved in the post-translational processing of the propeptide that is encoded by the POMC gene. Splicing of the propeptide results in the generation of adrenocorticotropin,  $\beta$ -lipotropin,  $\alpha$ -,  $\beta$ - and  $\gamma$ -melanocyte-stimulating hormones. Beside the mutations of the nuclear genome, several mutations in the mitochondrial DNA have been proved to be associated with obesity and related complications [33].

### **The gene polymorphism promoting the obesity-related metabolic complications**

In addition to genes - regulating restrictions of caloric intake (apetite), the numerous gene variants have been identified which appearance contribute to variation in lipid metabolism,

termogenesis, adipose tissue differentiation, immuno-inflammatory process, insulin resistance/ predispose to diabetes, thromboembolism etc. Examples of such genes are given in Table 3.

**Table 3.** Examples of genes with common variations associated with obesity-related metabolic disturbances. Adapted from references 54, 55, 9 and10

<b>Pathway</b>	<b>Mechanism</b>	<b>The gene/protein polymorphisms</b>
<b>Atherosclerosis</b>		
Lipid transport and metabolism	Plasma concentration of lipoprotein, reverse cholesterol transport effectiveness	<i>APOA-I, APOA-II, APO-AIV, apo(a), APOB, APOC-II, APOC-III, APOC-IV, APOD, APOE, APOH, APOJ, CETP, PLTP, MTP, FATPI, FABP2, LDLR, LRP, SR-BI, VLDLR, LPL, HL, LCAT, PON1, PON2, ABCA1</i>
<b>Hypertension</b>		
Renin-angiotensin system	Angiotensin II; vasoconstriction	<i>AGT, ACE, CYP11B2</i>
Sodium transport/metabolism	Sodium retention	<i>EnaC, adducin, 11b-hydroxysteroid dehydrogenase</i>
G-proteins	G-linked receptors activity	<i>GNAS1, adrenergic receptors</i>
Endothelium associated factors	Endothelial dysfunction	<i>iNOS, eENOS, tPA, PAI, VEGF</i>
<b>Hemostasis/Thrombosis</b>		
Platelet surface glycoproteins	Platelet adhesion and aggregation	<i>Glycoprotein Ia, Ib</i>
Coagulation factors	Thromboembolism	<i>Fibrinogen, prothrombin, factor V, factor VII, factor VIII, factor IX, factorXII, Factor XIII, thrombomodulin</i>
Thrombolytic system	Defective thrombolysis	<i>tPA, PAI-I</i>
<b>Type 2 diabetes</b>		
Energy metabolism regulators	Transcription factors	<i>PPARA, PPARG, HNF1A, HNF4A;m-Tor</i>
Insulin	Proteins and receptors	<i>Adiponectin, KCNJ11, CAPN10, TCF1, IRS1</i>



sensitivity/resistance		
<b>Inflammation</b>		
Inflammation factors	Expressed in adipocytes and blood cells	<i>TNFa, TNFb, TGFb1, TGFb2, IL1, IL1ra, CD14, P-selectin, E-selectin, PCAm-1</i>

\* **Abbreviations:** ABCA1- ATP-binding cassette, sub-family A (ABC1), member 1; ACE- angiotensin-converting enzyme; AGT angiotensinogen; apo(a)- apolipoprotein little a; APOA-I- apolipoprotein A-I; APOA-II- apo A-II; APOB- apo B; APOC-II- apo C-II; APOC-III- apo C-III; APOC-IV- apo C-IV; APOD- apo D; APOE- apo E; APOH- apo H; APOJ- apo J; CAPN10- calpain 10; CD14- monocyte differentiation antigen CD14; CETP- cholesteryl ester transfer protein; CYP11B2- cytochrome P450, family 11, subfamily B, polypeptide 2; EnaC- enactin; ENOS- endothelial nitric oxide synthase; FABP2- fatty acid binding protein 2; FATPI- Fatty acid transporter-1; GNAS1- Gs protein alpha subunit; HL- hepatic lipase; HNF1A- hepatocyte nuclear factor 1-alpha; HNF4A- hepatocyte nuclear factor 4-alpha; IL1- interleukin 1; IL1ra- interleukin-1 receptor antagonist protein; INOS- inducible nitric oxide synthetase; IRS1- insulin receptor substrate 1; KCNJ11- potassium inwardly-rectifying channel, subfamily J, member 11; LCAT- lecithin-cholesterol acyltransferase; LDLR- low density lipoprotein receptor; LRP- low density lipoprotein receptor-related protein; MTP- microsomal triglyceride transfer protein (large polypeptide, 88kDa); PCAm-1- platelet/endothelial cell adhesion molecule (CD31 antigen; PLTP- phospholipid transfer protein; PON1- paraoxonase 1; PON2- paraoxonase 2; SR-BI- scavenger receptor class B; TCF1- transcription factor 1, hepatic; TNFa- tumor necrosis factor alpha; TNFb- tumor necrosis factor beta; TNFb1- tumor necrosis factor beta-1; TNFb21- tumor necrosis factor beta-2; TPA- tissue plasminogen activator;

### Gene-gene interaction in polygenic model of obesity

The interpretation of genetic and environmental variances for multivariate and function valued phenotypes remain the main problem for estimation and interpretation. Deviation from health attributable to common complex disorders such as all components of obesity typically aggregate in families, but they do not segregate as Mendelian single genes. The new applications of known multidimensional statistical methods: such as principal component analysis (PC) and cluster analysis to evaluate the genetic variation consequences is presently used to solve this problem. PCs are implemented because they are statistically independent (orthogonal), describe the maximum amount of variation with the minimum numbers of parameters, and they are easy to be presented graphically. Even when the phenotype of interest has a large number of characteristics (dimensions in Euclides space), most of variations are typically associated with a small number of principal components and the principal component analysis may be used to visualize pattern of genetic variation. In this mode, the genetic principal components are calculated from an estimate of the full genetic covariance structure [74,75]. Direct estimation of the principal components reduces the number of parameters to be measured [76].



The another method- cluster analysis, similarly to principal component analysis, presents not only main pools of genes but also the hierarchy with different physiological significance [76,77,78].

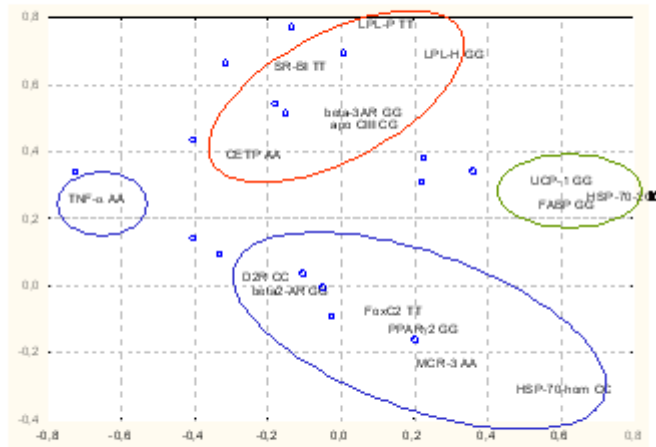


Figure 1: The principal component analysis segregate the genetic traits according to its similarities in formation of phenotype

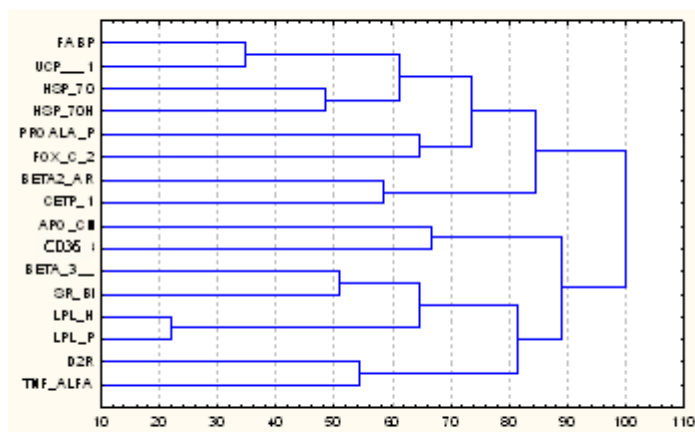


Figure 2: Cluster analysis. Lipid and postprandial lipemia parameters, and body mass index as dependent variables and age, sex, and each of the genetic variants as predictors were used

Figure 1 and Figure 2 show the examples of usage of both: PC-analysis as well as cluster analysis in our study. An application to the analysis of 14 most popular genetic traits and its phenotypic characteristics recorded during the familiar obesity study in South Poland is given. (our results, *Journal of Clin Chem Lab Med* 2007 in press).

Due to the implemented analysis it has been demonstrated that transcription factors (FOXC2 and PPAR- $\gamma$ 2) polymorphisms closely interact with each other and with the variability of genes regulating the carbohydrate metabolism. It is an argument for search of methods describing the interaction between the polymorphism of nutrient-sensitive transcription factors in regulating the metabolism of the body.

The risk of developing diet-dependent obesity is likely to involve interactions between many common but weakly penetrant genetic variants and numerous environmental and/or personal history factors. The construction of the diet - dependent disease risk model with both genetic and environmental/personal history measures to predict a specific risk of development of diseases in the early stage of life and at the stage of the absence of syndromes is very ambitious but unrealistic goal at this time. Thus the specific tests to validate genetic markers in aspects of nutrition are necessary.

The insulin output in response to the high carbohydrate or high fat diet seems to be the very promising parameter. We have demonstrated the difference in insulin output between the opposite allele of obesity risk that gene carries in reaction to standard high fat or high glucose overload (postprandial tests). The genotype dependent, nutrient induced insulin output ratio (NIOR), as a result of the different dietary load is presented in Fig 3. The introduction of NIOR allows for possible segregation of the patients with gene polymorphisms at risk to develop insulin resistance in relation to diet. (Fig 4) (our results, *Journal of Clin Chem Lab Med* 2007 in press)

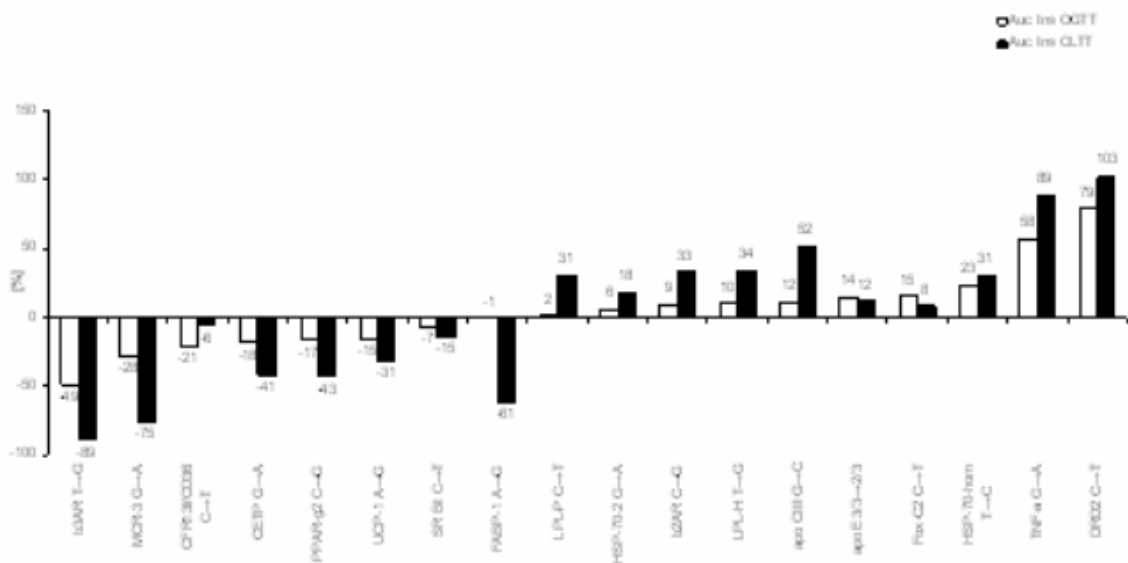


Figure 3. The ranking of the "candidates-genes" according to AUCinsins during OGTT. Such ranking is different for OLTT. The relative differences expressed as individual percent of AUCinsins determined by certain alleles according to formula:  $\text{The relative difference} = \frac{\{(\text{AUC}_{\text{cuc ilns}}(\text{BB}) - \text{AUC}_{\text{cuc ilns}}(\text{AA}))\}}{\text{AUC}_{\text{cuc ilns}}(\text{AA})} * 100$

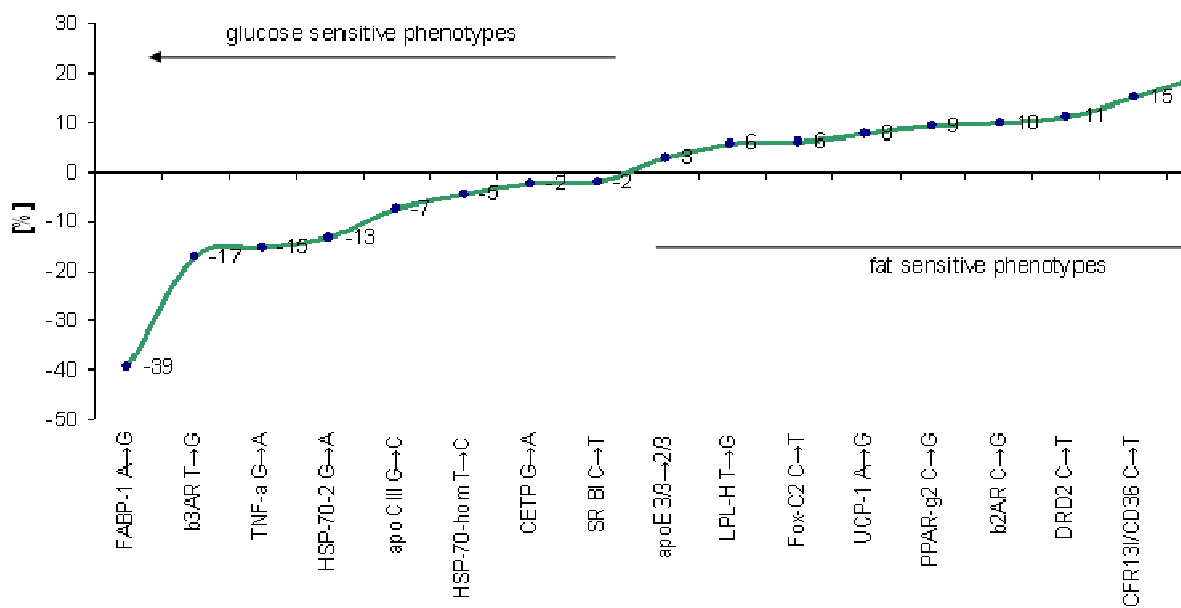


Figure 4: The ranking of the genotypic sub-groups according to NIOR differences between opposite allele carriers expressed as percent of change. The relative difference =  $\left[ \frac{(\text{NIOR (BB)} - \text{NIOR (AA)})}{\text{NIOR (AA)}} \right] * 100$

## Discussion

In the present paper we tried to present some of the examples of recent understanding of genetic background of obesity, gene-gene and gene-diet/environmental interactions. Development of complex markers, based on the integration of the functional tests and genomic technologies represent an exciting, technically challenging, approach for application of a new biomarkers at early stage of the disease.

The new scientific branch based on the recent, high throughput high-tech biotechnology and bioinformatics named "system biology" will be helpful in future to predict the gene-nutrient-environmental interaction and allows to introduce proper preventive therapy.

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