Abstract

Background The prevalence of obesity has almost tripled worldwide in the last four decades. Environmental and genetic factors are major contributors to the obesity epidemic. The main approaches used in the field of genomics during the last decade in order to understand the molecular mechanisms of obesity are mainly genome-wide association studies (GWAS) and, more recently, the determination of the molecular profile at all levels of the biological information. GWAS have discovered numerous SNPs and genes associated with obesity. *FTO* is the first and repeatedly verified gene, which contributes more than other genetic loci to the development of obesity. In addition, studies on differential gene expression patterns in adipose tissue have provided valuable, although limited, information on the expression profile of various genes while no meta-analysis of these data is available.

Aim To investigate the association of *FTO* gene polymorphisms with BMI and increased risk of obesity in Greek adults. So far, the association studies on *FTO* polymorphisms and obesity in Greece focus mainly on childhood obesity. Furthermore, in order to identify genes that are differentially expressed between obese and non-obese individuals as well as detecting gene expression signatures, pathways and networks associated with obesity, a meta-analysis of gene expression data in adipose tissue was performed.

Material/Methods The sample for the association analysis consisted of 203 individuals of Greek origin aged 68.53±9.38 years from the Diabetes Center of AHEPA University Hospital in Thessaloniki. Anthropometric measurements and biochemical characteristics were obtained and the individuals were categorized according to the World Health Organization's international standards based on BMI value, in non-obese (BMI<25), overweight (BMI≥25<30) and obese (BMI≥30). DNA samples were genotyped for the SNPs *rs9939609*, *rs9930506* and *rs3751812* of *FTO* gene using PCR-SSCP in combination with RFLP. The genotype frequencies were tested for Hardy-Weinberg equilibrium as well as the association of allelic and genotypic frequencies of the *FTO* with obesity. For the meta-analysis, gene expression data were retrieved from the GEO database. Three gene expression studies were selected and the samples were categorized in non-obese (BMI<30) and obese (BMI>30). Pre-processing of the data from different platforms as well as conversion of the probes IDs to gene names was applied. The meta-analysis was performed in collaboration with the Computational Genetics Laboratory of the Department of Computer Science and Biomedical Informatics of the University of Thessaly. Various methods of multiple testing corrections were used in the statistical analysis while PANTHER and STRING tools were used for bioinformatic analysis.

Results The study included 203 participants of which 24.6%, 28.6%, and 46.8% were non-obese, overweight, and obese, respectively. A statistically significant association was found between the *FTO rs9930506* 'G' allele and obesity (P=0.011) which remained statistically significant (Pc=0.022) after correction for multiple testing

(Bonferroni correction). Specifically, the risk 'G' allele showed a higher frequency in obese (53.7) compared to non-obese individuals (38.0) indicating that individuals carrying the 'G' allele have a 2.77 fold increased risk to be obese (OR: 2.77; 95% CI: 1.32–5.79; *P*=0.006). Similarly, the frequency of the GG genotype of *rs9930506* was found to be higher (0.29) in obese compared to non-obese individuals (0.20). Accordingly, the BMI of individuals with the GG genotype was higher. The analysis showed no association of *rs9939609* polymorphism with obesity (BMI) while SNP *rs3751812* does not satisfy the Hardy-Weinberg equilibrium.

The meta-analysis identified 821 differentially expressed genes (DEGs) in obese compared with non-obese (*p*-value<0.05) 108 of which form a network of protein interactions (PPIs). The network forms four distinct subnetworks (clusters): the first cluster contains ribosomal proteins, the second includes proteins responsible for mRNA processing and spliceosomal proteins, the third cluster comprises transcription factors and alternative splicing proteins and the fourth contains respiratory chain proteins. The enrichment analysis in the network identified 10 KEGG pathways. Furthermore, by all methods of multiple testing corrections, five statistically significant genes associated with obesity were identified, three of which, *NDUFA12*, *SFI1* and *SSB*, are down-regulated in obese individuals and two, *FAR2* and *LACE1*, are overexpressed.

Conclusions Association analysis showed that *FTO rs9930506* is a predisposition genetic marker for obesity in Greek adults while meta-analysis of gene expression data in adipose tissue revealed new candidate genes and pathways associated with obesity. It can be hypothesized that the down-regulation of *NDUFA12*, *SFI1* and *SSB* genes in obese is associated with obesity as a consequence of mitochondrial dysfunction (*NDUFA12*), ciliopathy (*SFI1*), protein synthesis and increased adipocyte volume (*SSB*) whereas the overexpression of *FAR2* and *LACE1* genes as a result of metabolic disorder (*FAR2*) and possibly mitochondrial dysfunction (*LACE1*). Further replication of the association analysis is needed in larger sample size in order to provide sufficient power in the study as well as functional analysis of both the genes identified in the meta-analysis and *rs9930506* polymorphism of the *FTO* gene.