OBESITY-Environmental Factors and/or genetic Influence

Genomic Structure, Mutational Analysis andPromoter Function of The Human Uncoupling Protein-2/-3 (hUCP2/hUCP3) Genes

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Today overweight and obesity has been recognized as a disease, a rapidly growing threat to health in an increasing number of countries worldwide, which is prevalent in both developing and developed countries and affects children and adult alike. What causes obesity? In scientific terms, obesity occurs when a person's calorie intake exceeds the amount of energy he or she burns. What causes this imbalance between consuming and burning calories? Evidence suggests that obesity often has more than one cause. Genetic, environmental, psychological, and other factors all may play a part. Obesity tends to run in families, indicating that it has a genetic cause. However, family members share not only genes but also diet and lifestyle habits that may contribute to obesity. Still, growing evidence points to heredity as a strong determining factor of obesity. In many studies of adults who were adopted as children, researchers found that the subjects' adult weights were closer to their biological parents' weights than their adoptive parents'. The environment provided by the adoptive family apparently had less influence on the development of obesity than the person's genetic makeup.

The role of genetic factors in obesity development is currently the focus of much research. Among the major breakthroughs in obesity research during the past several years has been discovered that the adipocytes hormone leptin (the product of OB gene, Zhang *et al.*, 1994) and leptin receptor (Maffei *et al.*, 1995), melanin concentrating hormone (Gonzalez et al., 1997), the melanocortin 4 receptor (Yeo *et al.*, 1998), urocortin (Zhao *et al.*, 1998;

Iino *et al.,* 1999), neuropeptide Y and its type 5 receptor (Pickavance *et al.,* 1999) and so on, all are involved in the control over the process of energy intake. In contrast, much less is known about the molecular basis of for determination of energy expenditure. However, the discovery and characterization of mitochondrial inner membrane ions carrier proteins – uncoupling proteins –represents a major breakthrough towards understanding the molecular basis for energy expenditure, and therefore likely to have important implication for the cause and treatment of human obesity.

UCPs considered as prime candidate genes involved in the pathogenesis of obesity. Due to the fact of limited abundance of UCP1 containing brown adipose tissue is unlikely to be involved in wieght regulation in adult large size animal and human living in a thermoneutral environment. Identification of UCP2 and UCP3 homologues in rodents implied as a major breakthrough towards discovery of the molecular basis for the energy expenditure. Therefore, What's the case in human? What are the potential functional roles of human UCP2 and UCP3 in energy metabolism and body weight regulation? How the expression of human UCP2 and UCP3 regulated? What are the possible implications of human UCP2 and hUCP3 for the pathogenesis and treatment of human obesity? Genetic studies in humans provide a method to test hypotheses about the biological role of specific genes, therefore this study focuses on human UCP2 and UCP3 genes function and transcriptional regulation.

Methods

The studies entirely base on current molecular Biology and Genetics means. Elucidation of the structural organization of human UCP2 and UCP3 genes by molecular cloning, sequences determination and exon / intron mapping. Identification and characterization of genetic variants in the 3'-UTR of human UCP3 gene using Rapid Amplification cDNA Ends (RACE) and RT-PCR (Reverse transcription-Polymerase Chain

Rection). Polymorphism analysis by Genotyping. Determination of functional properties of the 5'flanking and the promoter region of these two genes: elucidation the 5' Flanking Region of the hUCP2 gene by PCR-screening a human genomic library. Genome Walking of 5'-Flanking Region of human UCP3 Gene. Promoter analysis human UCP –2 /-3 utilizing pCAT-3 reporter gene system, transient transfection, CAT ELISA and protein determination

Results

Uncoupling proteins (UCP) are members of the family of mitochondrial anion carriers, which creates a pathway that allows dissipation of the proton electrochemical gradient across the inner mitochondria membrane thereby release storied energy as heat, without coupling to any other energy consuming process, uncoupling fuel oxidation from the conversion of ADP to ATP. This implies a major role of UCPs in energy metabolism and thermogenesis, which when deregulated are key risk factors in the development of obesity and other eating disorders. From the three different human UCPs identified by gene cloning, the human UCP1 (hUCP1) gene was assigned to human chromosome 4 (4q31) (Cassard *et al.,* 1990). Both UCP2 and UCP3 were mapped in juxtaposition to regions of human chromosome 11 (11q13) (Pecqueur *et al.,* 1999) that have been linked to obesity and hyperinsulinaemia (Norman *et al.,* 1997; Bouchard *et al.* 1997). At the amino acid level hUCP2 has about 55% identity to hUCP1 while hUCP3 is 71% identical to hUCP2.

Genomic organization

The human UCP2 gene spans over 8.7 kb distributed on 8 exons. The localization of the exon/intron boundaries within the coding region matches precisely that of the hUCP1 gene and is almost conserved in the recently discovered hUCP3 gene as well. The high degree of homology at the nucleotide level and the conservation of the exon /intron boundaries among

the three UCP genes suggests that they may have evolved from a common ancestor or are the result from gene duplication events.

Characterization the genomic structure of the human UCP3 gene implicates that hUCP3 gene spans about at least 7.5 kb distributed 7 exons and 6 introns, from which two mRNA transcripts are generated, UCP3L and UCP3S, which encode long and short forms of the hUCP3 protein differing by the presence or absence of 37 amino acid residues at the C-terminus. Mapping the boundaries of hUCP3L and hUCP3S transcripts. The potential transcription initiation site of hUCP3 mRNA was mapped at position -186 of the 5'-UTR by 5'-RACE (based the first base of the translational start codon ATG). 3'-RACE showed that the short form of hUCP3 is generated by incomplete transcription caused by the presence of a cleavage and polyadenylation signal (AATAAA) in intron 6 terminating message elongation. Alternatively, the elongation continues until another AATAAA signal in exon 7 of hUCP3 gene once the mRNA synthesis passes through the first polyadenylation signal.

Mutational analysis

Mutational analysis of the hUCP2 gene in a cohort of 172 children of Caucasian origin revealed a polymorphism in exon 4 (C to T transition at position 164 of the cDNA resulting in the substitution of an alanine by a valine at codon 55) and an insertion polymorphism in exon 8 consisted of a 45 bp repeat located 150 bp downstream of the stop codon in the 3'-UTR. The allele frequencies were 0.63 and 0.37 for the alanine and valine encoded alleles, respectively, and 0.71 versus 0.29 for the insertion polymorphism. The allele frequencies of both polymorphisms were not significantly elevated in a subgroup of children characterized by low Resting Metabolic Rates (RMR). So far a direct correlation of the observed genotype with (RMR) and Body Mass Index (BMI) was not evident.

Promoter Analysis

To analyze promoter function and regulatory motifs involved in the transcriptional regulation of UCP2 gene expression, 3.3 kb of 5'flanking region of the human UCP2 gene have been cloned by PCR-screening a human genomic library. Utilizing 5'-RACE, the potential transcription initiation site of hUCP2 mRNA has been identified to localize at position –364 of the 5'-UTR based the first base of the translational start codon ATG. Sequence analysis showed that the promoter region of hUCP2 lacks a classical TATA or CAAT box, however appeared GC-rich resulting in the presence of several Sp-1 motifs and Ap-1/-2 binding sites near the transcription initiation site. Functional characterization of hUCP2 promoter-CAT fusion constructs in transient expression assays showed that minimal promoter activity was observed within 65 bp upstream of the transcriptional start site (+1). 75 bp further upstream (from nt –141 to –66) a strong *cis*-acting regulatory element (or enhancer) was identified, which significantly enhanced basal promoter activity. The regulation of human UCP2 gene expression involves complex interactions among positive and negative regulatory elements distributed over a minimum of 3.3 kb of the promoter region.

To get insight into the mechanisms regulating human UCP3 expression, 5 kb of the 5'-flanking region of the hUCP3 gene were cloned and characterized by genome walking. The promoter region contains both TATA and CAAT boxes as well as consensus motifs for PPRE, TRE, CRE and muscle-specific factors like MyoD and MEF2 sites. Functional characterization of a 3 kb hUCP3 promoter fragment in multiple cell lines using a CAT-ELISA identified a *cis*-acting negative regulatory element between - 2983 and -982 while the region between -982 and -284 showed greatly increased basal promoter activity suggesting the presence of a strong enhancer element. Promoter activity was particularly enhanced in the murine skeletal muscle cell line C_2C_{12} reflecting the tissue-selective expression pattern of UCP3.

Future studies should be directed to clarify the potential significance these elements may have on regulation of hUCP2 / hUCP3 expression *in vitro* and *in vivo,* as well as to the identification of interactions between transcription factors and individual regulatory motifs localized in the 5' flanking region of hUCP3 and the entire 7 kb intergenic region between the UCP2 and UCP3 locus on human chromosome 11q13, in order to identify the underlying mechanisms orchestrating human UCP 2 and UCP3 genes transcription.

Conclusion

In conclusion, the discovery of the uncoupling proteins could be a breakthrough in understanding the complex mechanisms regulating energy expenditure and has given new stimuli for research in this field. The results so far strongly suggest a role for the UCPs in energy balance and obesity.

The genomic structure of the human UCP2 and UCP3 genes show great homology to the other known members of this family of mitochondrial carrier proteins; To get more definitive proof that UCPs are involved in regulating basal metabolic rates, and thus weight gain or loss,Analysis of the underlying mechanisms as regards regulation of UCP-2/-3 expression by promoter functional characterization in and cell culture determined regulatory motifs of tissue-specificity, hormone regulation and cis-/trans- acting elements in the upstream regions of UCP genes, which revealed the expression of UCPs is mainly controlled at the trancriptional level, and is positively regulated by the sympathetic nervous system CCAAT/enchancer-binding protein beta (C/EBP-b) plays a role as transcriptoinal activator of UCP genes, and peroxisome proliferator activated receptor(PPAR) gamma is also involved transcription regulation of UCPs via its ligands free fat acids, those basic studies will be on to find drugs that can combat obesity by tuning up the activity of the UCP proteins. If the level of uncoupling proteins could be slightly increased

1%-2%, then fat oxidation and thermogenesis would increased, and that could boost the resting metabolic rates of millions of people and whittle away their days of perpetual dieting.

Human uncoupling proteins have currently been in molecular basis of the research on energy expenditure, particularly nonshivering thermogenesis. Still, several Key questions are proposed and challenged in the near future, what are the molecular and physiological similarities and dissimilarities among UCPs? What are mechanisms of action? Are the other functions for UCP2 and UCP3? Finally, numerous different complex and diverse factors can give rise to a positive energy balance, Human obesity is a result of the interaction between a number of these influences, rather than any single factors acting along.

Because obesity prevalence continues to increase sharply as people approach the new century, today the challenge to scientists and public health worker in this area has never been greater.