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Rare Genetic Diseases with Human Lean and/or Starvation Phenotype Open New Avenues for Obesity and Type II Diabetes Treatment

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Abstract: Treatments of obesity and type II diabetes target often gene functions involved in appetite-satiety, fat and carbohydrate metabolism or thermogenesis. None of these, have provided efficient drug therapy to a large number of genes involved in weight and energy management, the redundancy of biochemical pathways and the environmental factors. Here I discuss a new approach based on studies of genes/proteins that are associated with human "lean or starvation" phenotype that became very rare in the course of evolution. This approach has led to the identification of the congenital enteropeptidase deficiency gene and the Anderson's Disease gene as a potential targets for obesity and type II diabetes treatment. The advantages of these targets are: 1) they are expressed exclusively in the intestine; 2) they are peripheral targets as opposed to systemic targets; 3) they are not redundant targets. These targets open new hopes for the development of novel drugs for the treatment of common metabolic syndrome.

Keywords: Diabetes treatment, drug discovery, lean phenotype, metabolic disorders, obesity, starvation phenotype, type II diabetes.

INTRODUCTION

Obesity and overweight are important risk factors for diabetes, hyperlipidemia and cardiovascular diseases leading to reduced life expectancy [1]. The prevalence of obesity is constantly increasing and it is estimated that in 2015 it will reach values ranging between 20 to 30% worldwide depending on the study and the numbers are even bigger in US [2]. Several hundreds of genes are involved in obesity [3] and the estimation is that one quarter of our genome is involved in weight management and energy metabolism [4]. Obesity and type II diabetes are complex traits, or phenotypes. They are complex compared to other phenotypes since most phenotypes do not change during life time, such as eye color, blood groups etc, while diabetes and/or obesity are variable phenotypes that are influenced by our genetics and the environment and therefore vary in population and along the individual lifespan. Actually type II diabetes and obesity are considered as life style disorders and they can be corrected or reverted by changing life style namely regime and exercise [5, 6].

During the French-German war (in 1870) when France lost the Alsace-Lorraine area, at that period Paris was besieged by the German army and there was a long period of famine. Apollinaire Bouchardat a French physician and pharmacologist working at the hospital Hôtel Dieu noticed that during the siege, there was a reduced incidence of diabetes in adult (onset diabetes) while diabetes in young (juvenile diabetes) was not affected, what was later known as Non Insulin Dependent-Diabetes Mellitus (NIDDM) and Insulin-Dependent Diabetes Mellitus (IDDM) respectively and today named type II and type I diabetes [7]. Bouchardat, at that time, was the first to introduce a systematic regimen in obese people with diabetes [7]. Weight reduction and increased exercise resulted in substantial improvements in their metabolic control, since that time Apollinaire Bouchardat has been considered as the father of diabetology. Even today, the first choice for type II diabetes treatment, when a patient is first diagnosed with hyperglycemia, is life style change, regime and exercise, in order to achieve weight reduction. Even a small weight reduction significantly improves glucose level in the blood and health conditions [5, 6, 8].

WHY DID HUMANITY EVOLVED TO BE OBESE?

Understanding why we evolved to be obese can help us identify and choose the right target for the treatment of obesity and type II diabetes. According to Darwinian natural selection, evolutionary change occurs through variations between individuals; some variations give the individual an extra survival probability. Individuals with characteristics which increase their probability of survival will have more opportunities to reproduce and their offspring will likely benefit from the heritable, advantageous character. As a consequence over time, these variants will spread through the population. Obesity or overweight characteristics present a paradox in terms of natural selection, since an obese individual did not have any obvious selection advantage. Obese individuals had a hard time to move fast as hunters or to escape danger as a prey. Moreover, today we know that obesity is tightly linked to diabetes, hyperlipidemia, hypertension and cardiovascular diseases [9] that reduce even more the evolutionary fitness of an individual. The solution to the paradox of obesity lay in the efficiency metabolizing and extracting energy from the digestive system. On an evolutionary timescale, when food was often scarce and hard to

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achieve, there was a natural advantage to possess efficient genes for extracting (from gastro intestinal tract) and metabolizing energy. The little left over of energy would be stored as lipids in order to survive the period of scarcity [10]. In our days, as food is abundant and our lifestyle is sedentary, these assets (efficiency and storing) have become a disadvantage. The natural selection for the efficient metabolic genes coupled to abundant food and a sedentary life resulted in an increase in overweight and obesity in most populations. Thus, obesity is a consequence of our genetic make-up, namely the natural selection of an efficient metabolism that clashes with the present sedentary life style and food abundance [11]. It would be wonderful if we could go back in evolution to have inefficient genes that for instance would allowed us to enjoy eating 3000 kCal and to extract and metabolize only half of it.

WHY OBESITY AND TYPE II DIABETES ARE TIGHTLY LINKED?

Weight management is estimated to be controlled by thousands of genes [3, 4, 10]. These weight management genes are involved within the central nervous system (CNS) to control appetite, satiety and thermogenesis, and all cells use metabolism of carbohydrate, fat and proteins, to generate biologically useful energy via glycolysis, Krebs cycle and phosphorylation oxidation mechanisms. Crude, ingested energy (fat, carbohydrates & proteins) in the end of a long metabolic process is transformed into ATP, an immediately available fuel for every cellular pathway in the body (Fig. **1**). Excess of crude energy is transformed into fatty acids and stored in adipose tissues [12]. However, one should keep in mind that the storage space in adipose tissue is finite. When that space is filled, the excess of crude energy is found in the blood as glucose (hyperglycemia), lipids (hyperlipidemia) or as ectopic fat accumulation, including the viscera, heart, and vascular system [12, 13]. Therefore, loosing weight, through diet or exercise, will free storage space in the adipose tissue and will revert the hyperglycemia, hyperlipidemia and the ectopic fat accumulation. Exercise and diet are the first choice for obesity and type II diabetes treatment. This observation was strengthened by the discovery of the mechanism of action of thiazolidinediones (TZD) compounds. This family of molecules is composed of activators of the Peroxisome Proliferator- Activated Receptors-γ (PPAR-γ) nuclear receptors. PPAR-γ receptors form heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate the transcription of various genes to stimulate adipocytes proliferation (adipogenesis) and lipid uptake [14-16]. Generation of more storage space by adipogenesis and lipid uptake by activation of PPAR-γ results in weight gain (one of the major side effects of this medication) and a reduction in blood glucose level [17-19]. In summary, in order to reduce blood hyperglycemia either we choose to partially empty the adipose tissues from their fat via diet and exercise or we increase the numbers of adypocite using PPAR-γ agonist such Troglitazone [20]. Needless to mention is that the first is preferable.

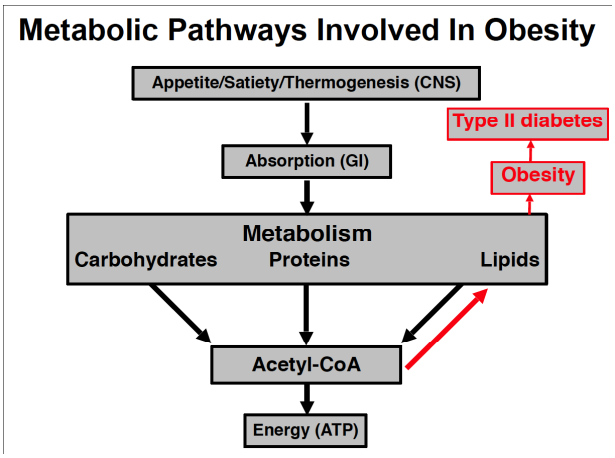

Fig. (1). Metabolic pathways involved in energy formation, obesity and type II diabetes.

BIG DATA AND METABOLIC DISORDERS

Sequencing of the whole human genome [21, 22] in 2001 was estimated to cost $100 million. Today the cost of sequencing the whole human genome is less than $10,000 per genome and as this price continues to drop, it will soon reach the psychological threshold of $1000/genome [23]. A huge and constant effort is done by the scientific community, governments, funding agencies and international consortia to identify new gene targets for metabolic syndrome using high throughput DNA-sequencing and genome wide association studies (GWAS). While these studies have provided insight into the nature of human sequence variation, it is not known at present whether these variations are truly significant and how much they contribute to a disease. It is widely accepted that most complex, common disorders such as diabetes and obesity are the results of the combined effects of multiple genes and non-genetic environmental factors. Therefore, it is likely that sequence variation alone will not be sufficient to predict the risk of developing a given complex disease [24]. A genome wide association study (GWAS) that was launched in a British population [25] included 2,000 individuals for each of the 7 major diseases including type II diabetes and a shared set of 3,000 controls. In this study 3 genes were identified with a strong association with type II diabetes, PPAR-γ, KCNJ11 and TCF7L2. Among these 3 genes only PPAR-γ was investigated as a target for type II diabetes treatment. The drugs developed against this gene target, known as Thiazolidinedione (TZD), displays major side effect as discussed above. In another independent GWAS, two diabetes-susceptibility genes were identified: a common variant of the FTO gene and the MC4R gene that predisposes to diabetes through an effect on the body mass index [26-28]. The HapMap (haplotype map) consortium started in 2002 and included several nations aims to identify and catalog the genetic similarities and differences in human. Using the information in the HapMap, researchers will be able to find genes that affect health, disease, and individual responses to medications and environmental factors including obesity and type II diabetes [29, 30]. The primary approach of the Genetic Investigation Anthropometric Traits (GIANT) consortium [31] has been meta-analysis of genome-wide association data and other large-scale genetic data sets. Anthropometric traits that have been studied by GIANT include body mass index (BMI), height, and traits

related to waist circumference. So far, the GIANT consortium has identified common genetic variants at hundreds of loci that are associated with anthropometric traits. In a recent study which included 263,407 individuals of European ancestry, 7 new loci (HNF4G, RPTOR, GNAT2, MRPS33P4, ADCY9, HS6ST3 and ZZZ3) for clinical classes of obesity were identified but their function and contribution to obesity and type II diabetes remain to be determined [32]. More recently the 1000 Genomes consortium which was launched in 2008, has sequenced the genome of about 2500 individuals. The goal of this consortium is to identify variants of rare and simple traits associated to diseases such as Cystic fibrosis, Hundington, Tay Sachs etc. A second goal is to identify common genetic variants of complex traits such as diabetes and obesity. Part of the results were recently published and describe 38 million SNPs, 1.4 million short insertions and deletions and more than 14,000 larger deletions [33]. Among all these consortia and many others, none of the genes or variants that have been linked or associated to obesity and/or type II diabetes was satisfactory for drug development against obesity, type II diabetes and hyperlipidemia treatment. Nonetheless, the above approaches and the variants identified thus far may provide a starting point for further investigation and might facilitate our understanding of the mechanism of these common metabolic disorders.

RARE GENETIC DISEASES WITH STARVATION PHENOTYPE.

The high redundancy of genes involved in energy management makes it unlikely that obesity will ever be controlled by targeting just one of those genes unless such a gene target is associated with a lean or starvation phenotype. In an earlier study we proposed that the identification of novel targets for treatment of obesity should aim to identify critical, non redundant genes that are involved in energy absorption, and that are associated with a «lean or starvation phenotype» [10]. If a monogenic slimness disease or a phenotype resulting in a deficiency in energy absorption could be identified, then the implicated gene would be likely to play a critical role in the phenotype, and it could be a potential target for new anti-obesity drugs especially if it were not compensated by other mechanisms. Such a gene would indeed be a more appropriate target for obesity treatment than the obesity genes in obese patients [34]. Among the genetic diseases associated with a lean human phenotype that have been described in the scientific literature, we will discuss the Anderson's Disease or Chylomicron Retention Disease [35] and Congenital enteropeptidase deficiency [36].

CONGENITAL ENTEROPEPTIDASE DEFICIENCY

Congenital Enteropeptidase deficiency is an extremely rare recessive inherited disorder: so far only few cases have been reported in the scientific literature [36]. Several mutations within the gene were described resulting in no EP activity [37-40]. This was confirmed in a later study that showed that mutations in the EP gene underlie the molecular cause of congenital EP deficiency and as a consequence a starvation phenotype is observed [41]. The pathology is successfully treated by pancreatic enzyme replacement or by administration of dietary protein hydrolysate [42-44]. In light of the foregoing, EP activity may provide a selective and efficient target for treating obesity and type II diabetes. *In vivo* testing of a novel borolysine-based EP inhibitors validates EP target for the treatment of obesity [34]. The EP is peripheral acting target that is reminiscent of the pancreatic lipase (PL), a valid target in the market for almost twenty years with commercialized drug under the name of Alli, Xenical or Orlistat. Partial inhibition of the EP will reduce the efficiency of energy absorption through the Gastro Intestinal (GI) tract. Reducing the daily absorption of energy deriving from proteins and fatty acids by 15 to 20% should have a significant impact on weight management in the long term. Furthermore, such a modest level of inhibition of both fatty acid and protein absorption is likely to trigger few, if any, side effects, such as diarrhea [34, 45].

ANDERSON'S DISEASE OR CHYLOMICRON RETENTION DISEASE (CMRD)

Three human genetic diseases have been described with very similar phenotypes: abetalipoproteinemia, hypobetalipoproteinemia, and chylomicron retention disease. The genetic causes of two of these diseases, abetalipoproteinemia and hypobetalipoproteinemia, have been elucidated. In abetalipoproteinemia a frameshift mutation in the microsomal triglyceride transfer protein (MTP) gene was described that results in complete absence of the MTP and its activity. This mutation therefore prevents formation and secretion of apoB containing lipoproteins, and consequently neither apoB100 nor apoB48 proteins are detected in the plasma of these patients [46, 47]. Hypobetalipoproteinemia is a heterogeneous disease in which different mutations in the apoB gene result in truncated apoB proteins of different lengths. So far a total of 25 mutations causing hypobetalipoproteinemia have been described in which most of the mutations are either nonsense mutations or frameshift mutations that result in a premature stop codon [48, 49]. The mechanism by which the production of these truncated apoB species results in hypobetalipoproteinemia is unclear. The third of these human genetic diseases with similar phenotypes, chylomicron retention disease, first described more than 50 years ago by Anderson has remained an enigma. This disease is characterized by chronic diarrhea, fat malabsorption, and failure to thrive and these are less severe than in abetalipoproteinemia and hypobetalipoproteinemia [50]. The disorder appears to follow an autosomal recessive pattern of inheritance. Plasma analysis of these patients shows complete absence of chylomicrons and apoB48 in the plasma. Inhibition of chylomicron formation and absorption from the intestine will result in inhibition of dietary fatty acids and cholesterol absorption which will be beneficial in long term treatment of obesity and type II diabetes. Many genes are involved in chylomicron formations and absorption; some of them are mentioned above. However, the SAR1B gene [51] was shown to be involved in chylomicron retention disease. Human apoB is the major apolipoprotein of the triglyceride-rich lipoproteins (VLDL, LDL and chylomicrons) and the gene is expressed in both liver and intestine. The liver produces a protein containing 4536 amino acids referred to as apoB100, whereas in the intestine the same gene encodes a smaller proteins containing 2152 amino acids referred to as apoB48 and which is co-linear with the amino terminal half of apoB100. ApoB48 is produced as a result of posttranscriptional editing due to the

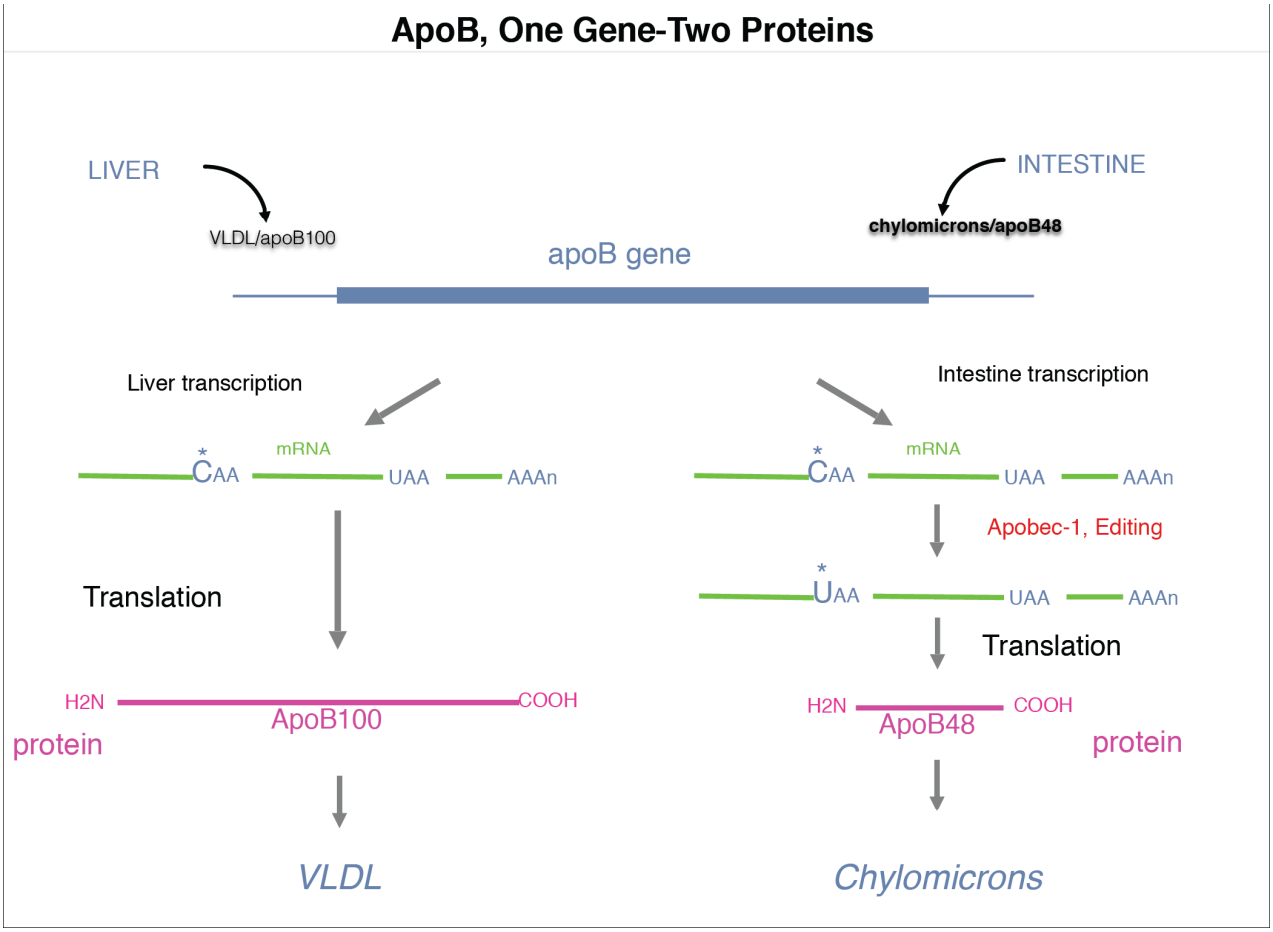

Fig. (2). Apobec-1 enzyme deaminate C to U in apoB mRNA at position 6666 to generate a stop codon in the intestine for the generation of apoB48 protein as part of chylomicrons.

activity of the apoB mRNA editing protein (apobec-1) which deaminates cytidine at position 6666 to uridine in the apoB mRNA and produces a UAA in- frame stop codon [52] (Fig. **2**).

Another candidate gene for Anderson's Disease and potential target for obesity treatment could be apobec-1 since it is exclusively expressed in the intestine. It is involved in the editing of the main protein of chylomicrons and only the chylomicrons containing apoB48 are missing in Anderson's disease patients, while VLDL containing apoB100 are present in plasma. Furthermore, target disruption of mouse *apobec-1* abolishes apoB mRNA editing activity and no apoB48 is found in the plasma [52, 54]. Absence of apoB48 is the major phenotype in Anderson's disease patients. It should be noted that a defect in the editing protein may be due to a mutation in the cytidine deaminase itself or in its associated accessory factors which are absolutely essential for the *apobec-1* activity [55]**.** Alternatively, point mutations within the mooring sequence in the apoB gene, and other mutations within the highly conserved sequence motif surrounding the CAA position 6666 will also abolish or severely reduce the editing activity. It is not excluded, however, that Anderson's disease might be due to a problem in the secretory pathway via other cofactors and/or proteins different than MTP. In any case whether apobec-1 is the missing gene in Anderson's disease or any other genes, these missing genes might be an excellent target for obesity and type II diabetes treatment since the missing genes, apparently, are exclusively expressed in enterocytes and only chylomicrons are missing in Anderson's disease [56, 57].

Recently, transgenic rabbits expressing permanently and ubiquitously a small hairpin RNA targeting the rabbit apobec-1 mRNA were generated. These rabbits exhibited a moderate but significantly reduced level of apobec-1 gene expression in the intestine, a reduced level of editing of the APOB mRNA, a reduced level of synthesis of chylomicrons after a food challenge, a reduced total mass of body lipids and finally presented a sustained lean phenotype without any obvious lipid and vitamin deficiency. This strengthens the hypothesis that the apobec-1 gene is a valuable target to treat obesity, type II diabetes and hyperlipidemia [58].

CONCLUSIONS

Extraordinary progress in our understanding obesity and type II diabetes has been made during the past two decades and many genes associated with metabolic syndrome have been identified and their function elucidated, yet it remains

challenging to identify new validated targets for obesity and type II diabetes treatment mainly due to the side effects associated to the target [10]. The vast majority of targets described so far in the scientific literature were abandoned at early stage of research or during their development. Moreover, the small numbers of molecules commercialized for obesity treatment were retracted from the market a short time after their introduction and those which are still available suffer from low efficacy [10]. None of these gene targets was shown to be linked or associated with the lean phenotype. We believe that in order to successfully treat obesity and type II diabetes one should target genes linked with a "lean or starvation human phenotype". This approach has led to the identification of enteropeptidase and apobec-1 as a potential target for obesity and type II diabetes treatment. *In vivo* inhibition of enteropeptidase with pseudopeptides has a direct effect on weight management due to the inhibition of fatty acids and proteins absorptions (34). Enteropeptidase is thus an excellent target for obesity treatment. Recently, we have also validated apobec-1 as target for obesity treatment by shutting down the gene in rabbit using RNAi technology [58]. These rabbits exhibit a lean phenotype when compared to controls. We remain confident that anti-obesity agents that target genes or proteins associated with a "lean or starvation human phenotype" would greatly increase the probability of successful treatment, as compared to those that target genes associated with an obese phenotype. Moreover, several interesting lean phenotypes associated with single gene mutations should be studied. Thus other gene/protein targets may be discovered and validated using reverse genetics on a lean or starvation phenotype.

CONFLICT OF INTEREST

The author confirm that this article content has no conflicts of interest.

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