Target discovery in metabolic disease

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The prevalence of metabolic diseases is taking on epidemic proportions and poses a serious threat to human health. Current treatment options have proven insufficient to cope with obesity and diabetes because they rarely restore normal metabolism and thus leave patients exposed to life-threatening complications. Successful management of these diseases depends on novel, improved therapeutic strategies targeting early intervention in disease progression. Discovery of novel metabolic disease targets has been hampered by the complexity of contributing environmental and genetic factors, as well as the need for potent but safe treatments suitable for chronic diseases. Genomic approaches are excellent tools to manage genetic complexity and have been applied successfully to identify candidate target genes that will lead to the development of novel therapies for metabolic diseases.

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Metabolic diseases affecting energy homeostasis such as obesity, type 2 diabetes (T2D) and the metabolic syndrome are a major challenge for healthcare systems worldwide because they are strongly associated with several major health risk factors [1–4]. Currently marketed drugs, which are aimed at achieving long-term control of body weight and blood glucose levels, largely target symptoms with limited efficacy and considerable side effects that raise compliance issues [5,6]. Development of more effective drugs for metabolic diseases requires the identification of mechanisms and targets that have the potential to be developed into improved therapeutic but also preventative strategies.

Sequencing of the human genome combined with technological development of large-scale genomic approaches has made it possible to systematically investigate genetic factors for their involvement in human diseases. Genomic approaches such as large-scale gene expression analysis, functional screens in model organisms, and human genome scans for susceptibility genes have led to the identification of countless candidate drug targets for most major indications including metabolic diseases. Owing to the wide-ranging technological spectrum of these approaches, there are tremendous differences in resources and timelines they require as well as the results they have produced so far. This review focuses on the contributions of these genomic technologies to target discovery in obesity and T2D, with an emphasis on applied strategies as well as emerging principles rather than individual results.

Expression profiling

So far, expression-profiling efforts have relied to a large extent on technologies analyzing the composition of complex mRNA samples and, less frequently, protein samples. Widely used approaches for assessing the composition of mRNA pools or differences between them are differential display, subtractive cloning or, with increasing popularity, DNA microarrays carrying probe sets that cover essentially complete genomes [7]. Tissues and organs that are of particular interest in metabolic diseases include adipose tissue, muscle, liver, pancreas and the hypothalamus, which have pivotal roles in the regulation of energy storage, maintenance of blood glucose homeostasis and appetite control. Below, selected expression profiling studies are presented to exemplify how these technologies have been applied to address peripheral mechanisms of energy homeostasis, which could provide the underlying cause for several metabolic diseases (Table 1).

Adipose tissue

Adipose tissue is not only the primary organ responsible for storage of excess energy but also fulfils important endocrine functions that affect peripheral and central mechanisms involved in maintaining energy homeostasis. First attempts to define the composition of adipose tissue on the mRNA and protein level have been carried out by Gabrielsson *et al.* [8] and Sanchez *et al.* [9], respectively. Further experiments investigated molecular differences

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Table 1. Expression profiling approaches in metabolic disease

Experimental details	Species	Technology	Refs
Adipose tissue			
Abdominal subcutaneous adipose tissue from non-obese individuals	Human	Microarray	[8]
White and brown adipose tissue from metabolically healthy mice	Mouse	Proteomics	[9]
Perirenal visceral versus subcutaneous adipose tissue	Rat	Microarray	[10]
Abdominal omental adipose tissue from non-obese individual (female)	Human	Microarray	[11]
Omental versus subcutaneous from obese individuals (male)	Human	Microarray	[12]
Visceral versus subcutaneous adipose tissue from obese individuals (male/female)	Human	Differential display	[13]
White adipose tissue from lean versus HFD induced obese mice	Mouse	Microarray	[16]
Adipose tissue from rats at the onset (7, 14, 21 days) of HFD-induced obesity	Rat	Subtractive cloning	[15]
Adipose tissue from genetically obese (ob/ob) versus HFD-induced obese mice	Mouse	Microarray	[21]
Adipose tissue from ob/ob versus HFD-induced obese mice	Mouse	Micorarray	[22]
Adipose tissue from lean versus ob/ob mice and leptin-treated versus food restricted	Mouse	Microarray	[18]
Adipose tissue from lean, obese and obese-diabetic mice	Mouse	Microarray	[17]
Omental adipose tissue from lean versus normotensive, normoglycemic obese individuals	Human	Microarray	[19]
Adipose tissue non-diabetic, insulin resistant versus diabetic individuals	Human	Microarrays	[20]
White adipose tissue versus brown adipose tissue	Rat	Microarray	[31]
Adipocytes			
3T3-L1 preadipocytes (day 0) versus differentiated adipocytes (day 6)	Mouse	Micorarray	[25]
3T3-L1 *in vitro* differentiation time course (24 hours, 4 days, 1 week)	Mouse	Micorarray	[26]
3T3-L1 early *in vitro* differentiation time course (0, 2, 8, 16, 24 hours)	Mouse	Microarray	[27]
3T3-L1 differentiation time course versus primary predipocytes and adipocytes from WAT	Mouse	Microarray	[28]
Primary preadipocytes versus adipocytes from subcutaneous adipose tissue (average BMI 29)	Human	Microarray	[29]
NIH-3T3 fibroblasts stably transfected with PPAR-γ versus vector transfected	Mouse	Subtractive cloning	[50]
3T3-L1 preadipocytes differentiated in the presence of PPAR-γ agonist (rosiglitazone)	Mouse	Differential display	[49]
Muscle			
Muscle tissue from STZ-treated (insulin deficient) diabetic mice	Mouse	Microarray	[33]
Gastrocnemius muscle from metabolically healthy mice	Mouse	Proteomics	[9]
Muscle tissue from human skeletal muscle during hyperinsulinemic clamp	Human	Microarray	[34]
Skeletal muscle of type 2 diabetes patients during intense insulin treatment versus untreated	Human	Microarray	[35]
Muscle of type 2 diabetic patients (insulin-resistant) versus non-diabetic (insulin-sensitive)	Human	Microarray	[36]
Muscle from non-diabetic, insulin-resistant versus non-diabetic, insulin-sensitive	Human	Differential display	[38]
Muscle from obese non-diabetic, insulin-resistant versus non-diabetic, insulin-sensitive	Human	Micorarray	[37]
Liver			
Liver tissue from metabolically healthy mice	Mouse	Proteomics	[9]
Liver tissue from obese diabetes-susceptible versus obese but diabetes-resistant mice	Mouse	Microarray	[42]
Liver tissue from STZ-treated diabetic mice	Mouse	Microarray	[41]

Table 1. Continued

Experimental details	Species	Technology	Refs
Liver (continued)			
Liver tissue from leptin-deficient mice, leptin-treated versus untreated	Mouse	Microarray	[40]
Liver tissue from lean and obese mice treated with PPAR-α and -γ agonists	Mouse	Proteomics	[52]
Liver tissue from rats treated with PPAR-γ agonist	Rat	GeneCalling	[51]
Pancreas			
Isolated pancreatic islets from metabolically healthy mice	Mouse	Proteomics	[9]
Developmental stages of pancreas development, isolated islets, insulinoma	Human	EST sequencing	[7]
Fetal pancreas, isolated islets and insulinoma	Mouse	EST sequencing	[7]
Pancreatic tissue from partially ligated pancreatic duct mice (pancreas regeneration model)	Mouse	Microarray	[49]
Isolated and cultured human islets	Human	Microarray	[48]

Abbreviations: BMI, body mass index; HFD, high fat diet; STZ, streptozotocin; WAT, white adipose tissue.

between subcutaneous and visceral human adipose tissue [10–13]. The results indicate that adipose tissue is more complex than originally appreciated. In addition to simply storing and mobilizing lipids, adipose tissues produce and release metabolically active polypeptides or metabolites that can be involved in immunity (complement factors), endocrine function (leptin), metabolism (adiponectin, resistin), and cardiovascular control (angiotensinogen, plasminogen activator inhibitor-1) [14]. Furthermore, experiments investigating molecular differences between subcutaneous and visceral human adipose tissue strongly suggest that fat depots from various regions of the body are functionally diverse in the way they respond to endocrine factors, the adipokines they produce and whether or not they are able to carry out thermogenesis [10–13].

Other studies addressed changes in adipose tissue gene expression during the transition from a metabolically healthy state to increasingly diseased states. Li *et al.* [15] determined that genes involved in the expansion of non-adipose tissue are upregulated at the onset of diet-induced obesity (DIO) possibly driving the proliferation of preadipocytes. By contrast, Moraes *et al.* [16] looking at later stages of DIO, found genes involved in lipid metabolism and adipogenesis to be downregulated and inflammatory markers to be upregulated. Specifically, the observation that genes normally associated with adipocyte differentiation are downregulated in the obese state is supported by studies in genetically obese mice, strongly suggesting that adipose tissue of obese mice might not be fully functional and therefore contribute to the progression of the disease from an obese but non-diabetic state to the obese–diabetic state [17,18].

More recent studies carried out in humans comparing the insulin resistant but non-diabetic versus the diabetic state and the lean versus the obese state also provide evidence that there are significant changes in gene expression in diseased adipose tissue. For example, variations in gene expression in omental adipose tissue from normoglycemic, normotensive obese patients (BMI 37.3 kg m^{-2}) when compared to lean individuals (BMI 23.4 kg m^{-2}) are associated with a broad spectrum of biological processes. In obese patients, these changes include an increase in the expression of genes involved in lipolysis repression, mitogen-activated protein kinase signalling, angiogenesis and immune function, whereas there is a decrease in the expression of genes involved in lipolysis activation, preadipocyte proliferation and differentiation. Similarly, in the insulin resistant state, early and late markers of the adipogenic process are downregulated, lending further support to the hypothesis that adipose tissue in obese patients is functionally impaired [19,20]. Obesity is associated with a state of chronic, low-grade inflammation that appears to reside predominantly in adipose tissue and is characterized by macrophage infiltration. It is hypothesized that macrophages in adipose tissue might contribute to the elevation in circulating inflammatory markers, which have been linked to the development of insulin resistance [21,22]. Regardless of whether or not the initial inflammatory response emerges from the adipocyte and further propagates with the recruitment of macrophages or whether macrophages infiltrate the adipose tissue and subsequently initiate the inflammatory response themselves, treating inflammation of adipose tissue could provide an alternative mechanism for pharmacological intervention [23].

Targeting adipogenesis is a proven mechanism for improving insulin resistance, as demonstrated by thiazolidinediones (TZDs) such as Avandia (GlaxoSmithKline; http://www.gsk.com) and Actos (Lilly; http://www.lilly.com),

which are agonists of peroxisome proliferator-activated receptor (PPAR)-γ, a key driver of adipocyte differentiation and an important drug target for T2D. Identification of genes in related pathways with similar effects on adipogenesis or adipocyte function might provide alternative targets without the potentially target-related side effects observed with TZDs. The process of adipogenesis has been studied predominantly in the mouse 3T3-L1 preadipocyte cell line [24]. Various groups have analyzed gene expression during adipogenesis looking either at early or late stages of the differentiation process [25–27]. These studies revealed many functionally uncharacterized genes that are regulated during adipogenesis as well as differences in gene expression between human and mouse adipogenesis previously not appreciated [28,29]. It is of interest to note that in addition to PPAR-γ, several genes that are upregulated during adipogenesis are being pursued as potential drug targets. These include 11-β-hydroxysteroid dehydrogenase type-1 (11-β-HSD-1), probably the most advanced target in regards to drug development, but also fatty acid binding protein, hormone-sensitive lipase (HSL), and resistin, among others.

Another attractive mechanism, especially for the treatment of obesity, is to increase energy expenditure through activation of uncoupling proteins (UCPs). Whereas the physiological role of white adipose tissue (WAT) is to store excess energy in the form of fat, the main function of brown adipose tissue (BAT) is to use existing fat stores for thermogenesis via activation of UCP1 [30]. Identifying genes that are essential for efficient energy dissipation in BAT will lead to a better understanding of UCP-related pathways and components of these pathways could provide potential metabolic disease targets. Unami *et al.* [31] addressed this question through gene expression analysis of WAT and BAT identifying many differentially expressed genes which await further functional analysis.

Muscle

Similar to adipose tissue, skeletal muscle has a central role in metabolic diseases primarily because it is the major site of glucose disposal in response to insulin and thus the main site of insulin resistance in T2D. Various studies have investigated the transcriptional response to an insulin stimulus, identifying >150 insulin-regulated genes [32]. More recent *in vivo* studies analyzed the changes in gene expression in skeletal muscle of mice either lacking an insulin signal, during hyperinsulinemic euglycemic clamp, or untreated versus insulin-treated T2D [33–35]. Furthermore, in attempts to identify molecular mechanisms, and thus candidate drug targets, associated with insulin resistance in skeletal muscle, gene expression analysis was conducted in non-diabetic versus diabetic and insulin sensitive versus insulin resistant but non-diabetic human subjects [36–38]. All of these studies indicate that insulin action, or the lack thereof in skeletal muscle, leads to highly coordinated changes in gene expression in various metabolic pathways, including glucose, protein and lipid metabolism but also cell proliferation and survival. Despite the fact that the insulin pathway has been extensively investigated and many of its components are pursued in drug discovery efforts (e.g. protein tyrosine phosphatase-1b, glycogen synthase kinase-3, protein kinase B, SH2 domain-containing inositol 5-phosphatase), the complex intracellular signalling network involved offers many more targets with the potential to attack insulin resistance directly.

Liver

The liver has a central role in maintaining blood sugar levels by acting as a storage depot during periods of dietary carbohydrate excess and as a glucose source when carbohydrates are unavailable. Hepatic glucose production is inappropriately high in T2D primarily due to increased gluconeogenesis rather than glycogenolysis. The biguanide Metformin [Merck-Lipha (http://www.lipha.fr) and Bristol-Myers Squibb (http://www.bms.com)] is the only marketed agent targeting hepatic glucose production as its primary mode-of-action. Other protein targets that control hepatic glucose production that are currently being pursued (such as glucagon receptor, glycogen phosphorylase, glucocorticoid receptor, fructose-1,6-bisphosphatase, carnitine palmitoyltransferase-1, glycogen synthase kinase-3, glucose-6-phosphate translocase, adenosine A2B receptor and 11-β-HSD-1) are still in preclinical and clinical stages of investigation [39]. Further candidate target genes regulating the coordinated expression of key gluconeogenic and glycogenolytic enzymes, as well as other mechanisms, should certainly be among those genes identified in expression profiling efforts investigating the liver under metabolic stress, such as absence of an insulin stimulus or in the diabetic or obese state [40–42]. These studies revealed that a wide variety of genes involved in fatty acid metabolism are transcriptionally regulated in the diseased state, suggesting that hepatic fat metabolism might also offer potential targets for intervention.

Pancreas

Another important peripheral organ for the treatment of T2D is the pancreas, where primarily endocrine hormone-producing islets are targeted to secrete more insulin in response to elevated blood glucose levels. Although insulin secretagogues, including sulfonylureas, glitinides and meglitinides, are important therapeutic options, it is likely

that future drugs will target the preservation of β-cell function and β-cell mass rather than insulin secretion. In particular, the concept of targeting β-cells directly has received much support from work by Dor *et al.* [43], suggesting that terminally differentiated β-cells retain significant proliferative capacity in mice and have a significant role in maintaining β-cell mass. Glucagon-like peptide (GLP)-1 analogues such as exenatide (Eli Lilly) are in late stage clinical trials and have been shown to improve β-cell function and potentially even increase β-cell mass, making it likely that more targets that are directed at similar mechanisms will follow [44]. Dipeptidyl peptidase (DPP) IV inhibitors, which are currently in advanced clinical development [Novartis (http://www.novartis.com) and Merck (http://www.merck.com)], target essentially the same mechanism by preventing the degradation of endogenous GLP-1 [45]. Similar to the GLP-1 analogues, there is evidence indicating that a lack of DPP IV function might prevent streptozotocin-induced as well as high fat diet-induced β-cell apoptosis and potentially might even contribute to islet neogenesis [46,47]. Molecular drug target candidates are rare in this area and published reports about expression profiling approaches aimed at the identification of pancreatic drug targets are limited [7,9,48,49].

Expression profiling of disease-relevant pathways

Alternative target discovery approaches are aimed at the elucidation and dissection of highly disease-relevant pathways such as for PPAR-γ, leptin and insulin. Pharmacological treatment, genetic loss or gain of function studies in appropriate cell lines or tissues combined with differential gene expression analysis can lead to the identification of transcriptionally regulated components of specific signalling pathways, which might be suitable for therapeutic intervention. An example for studying insulin action was already presented in the study by Rome *et al.* [34]. PPAR-γ and -α agonists have been used *in vitro* and *in vivo* to elucidate ligand-induced changes in mRNA expression in adipose tissue and liver [50–53]. Although the main intention was to evaluate compound efficacy and potentially identify useful clinical markers, these studies also revealed unexpected changes in expression patterns, which could lead to a more comprehensive picture of these pathways. Finally, the leptin pathway is of interest because of the potent effects of leptin on food intake, energy expenditure and many other metabolic processes. Soukas *et al.* [18] administered leptin to wild-type and leptin-deficient mice to identify kinetically distinguishable gene clusters that are specifically regulated by leptin. The results strongly suggest that in addition to its central effect on food intake, leptin activates metabolic pathways that deplete adipose tissue, making the leptin signal transduction pathway a highly interesting target in peripheral tissues as well as the CNS. Thus, despite the fact that clinical trials with leptin have been disappointing in regard to energy expenditure and weight loss potentially due to an underlying leptin resistance in obese patients, components of the leptin signal transduction pathway should prove interesting drug targets in the future.

The limits of expression profiling

Most of the expression profiling experiments described above have led to the discovery of large numbers of regulated genes and gene clusters and therefore represent an excellent first step towards the identification of mechanisms associated with a certain physiological state, and thus potential metabolic disease targets. However, it is important to keep in mind that expression-profiling approaches have technical and conceptual limitations. Although the technologies are constantly improving, the majority of the presented studies provide neither a comprehensive picture regarding the transcriptional status of the complete genome nor an adequate representation of the dynamic range of transcriptional regulation. It remains a challenge, particularly for microarray-based studies, to decide at what point a gene should be classified as transcriptionally regulated because the dynamic range of detection is usually limited. It is also important to note that observed changes on the transcriptional level are not necessarily reflected on the protein level and that genes whose activity is primarily regulated on the post-transcriptional level will not be detected by RNA-based technologies. Furthermore, for all studies comparing the expression status of healthy and diseased tissues, it remains to be determined if transcriptional regulation of a particular gene is directly related to a primary defect or is rather the consequence of a prolonged pathophysiological state. For example, in patients with increased free-fatty-acid levels, the primary defect could be an increase in lipolysis, which in turn causes insulin resistance, or alternatively the primary defect could be insulin resistance, which leads to an increase in lipolysis. Such questions are probably addressed most easily in systems where it is possible to follow disease development and progression in detail, such as model organisms. Despite these issues, expression-profiling studies have provided a wealth of candidate genes, which have been associated with particular physiological functions or disease mechanisms. The future challenge will be to mine these data appropriately and select the most interesting genes for further functional validation. In summary, expression-profiling studies are highly valid approaches to identify novel mechanisms

involved in metabolic diseases as well as potential candidate target genes as demonstrated by several examples. However, in most cases they only represent a first step that requires in depth analysis of the generated datasets, as well as additional functional experiments to select high potential targets for further drug discovery efforts.

Human genome scans

Genome-wide scans for susceptibility genes are conducted with the use of genetic polymorphisms such as simple tandem repeats or single nucleotide polymorphisms, performing linkage analysis in usually related individuals with a certain disease and/or characteristic quantifiable trait associated with the disease. A large number of human genome scans have been performed and there are comprehensive reports detailing the results for susceptibility loci associated with T2D, obesity and the metabolic syndrome [54–56].

The emerging picture is one of high complexity, with potential susceptibility loci distributed all over the human genome except for the Y chromosome. Interestingly, several independent studies provide accumulating evidence that a significant number of loci are being linked to both T2D and obesity, strongly suggesting a common underlying genetic architecture [57,58]. Due to the inherent genetic complexity of multi-factorial diseases, the contributions of individual gene variants are usually modest and therefore hard to detect, especially if there is variation among analyzed patient populations and the screened patient numbers are relatively small. The problem of genetic complexity is compounded by the tremendous variability of encountered environmental factors (e.g. diet, job, exercise). Nevertheless, there is accumulating evidence for significant association of ~90 candidate genes with obesity. However, in many cases linkage only accounts for a small fraction of the patient population and in some cases might even turn out to be false positives. In the case of T2D, overall >25 susceptibility genes have been identified, at least 21 of which are responsible for maturity-onset diabetes of the young (MODY), now referred to as the monogenic and/or syndromic form, whereas only six genes have been associated with multi-factorial T2D. In monogenic T2D the primary defect is in insulin secretion and not adiposity combined with peripheral insulin resistance, which are hallmarks of multi-factorial T2D. The pathophysiological basis for multi-factorial T2D is not well established but clearly important contributing factors are peripheral insulin resistance coupled with a progressive impairment of insulin secretion. The progressive loss in β-cell function is becoming increasingly important for multi-factorial T2D, therefore, the borders between the monogenic and multi-factorial T2D are less well defined. Currently, there are six different MODY genes, accounting for 90% of MODY families, which predominantly affect insulin secretion and have been identified through a combination of positional cloning and candidate gene-based analyses. Overall, despite significant linkage of many loci with metabolic diseases, the only susceptibility gene identified by positional cloning efforts alone is clapain-10 (CAPN-10) for multi-factorial diabetes [59].

So far, in comparison to human genome scans, it has been more productive to screen patients for mutations in candidate genes that were initially identified by other means. This 'candidate gene' approach has been reasonably successful in linking mutations in at least 15 genes (β adrenergic receptors 2 and 3, guanine nucleotide binding protein, leptin receptor, leptin, melanocortin 4 receptor, glucocorticoid receptor, proprotein convertase subtilisin/kexin type 1, proopimelanocortin, PPAR-γ, PPAR-γ coactivator 1, protein phosphatase 1 regulatory subunit 3A, tumor necrosis factor, single-minded homolog 1, and uncoupling proteins 1–3) to obesity, as a dominant clinical feature. However, considering that several hundreds of biological candidate genes, previously implicated to be involved in β-cell, adipocyte, liver or other metabolic function, have been examined for their potential role in metabolic disease these are relatively few cases where good evidence for a contribution to the susceptibility of metabolic disease could be established [55,59]. In summary, genome scans have not contributed significantly to the identification of novel targets so far but are a valuable resource for confirming the relevance of target gene candidates. The combination of positional cloning efforts and association studies of candidate genes will deliver many more metabolic disease susceptibility genes, such as CAPN-10, and thereby significantly improve our understanding of the pathophysiological basis of metabolic diseases, and ultimately provide novel mechanisms and molecules to be targeted.

Functional screens for metabolic disease targets in model organisms

Functional screens in model organisms are a powerful approach to functionally assign genes to certain biological processes and are therefore well suited to identify key components of mechanisms controlling metabolic homeostasis. The first step usually involves mutagenesis of the genome via chemical, genetic or RNAi-based technologies, which is then followed by scoring of individual mutants or mutant lines for relevant phenotypes. Screens covering large portions of the genome have been performed successfully in fruit flies (*Drosophila melanogaster*), zebrafish and mice [60–62]. However, published reports of genome-wide

model organism screens specifically designed to identify metabolic disease phenotypes are still the exception although they are highly complementary to expression profiling and human association studies.

Mouse

The mouse is the most important model organism for target validation as demonstrated by the fact that knockout phenotypes for the 100 best-selling drugs correlate well with known drug efficacy [63]. This paradigm seems to hold true for metabolic diseases and is reflected in pipeline targets of the pharmaceutical industry (serotonin receptor 5-HT2C, cannabinoid CB1 receptor, cholecystokinin receptor, GLP-1 receptor, DPP IV) in the area of obesity and T2D that are currently in late stage clinical trials [64]. Although various approaches to undertake systematic genome-wide functional screens in the mouse have been initiated, none of these screens was specifically focused on metabolic phenotypes. Generally, mouse models with metabolic phenotypes have been generated by selective targeting of a particular gene, which was either previously implicated in metabolism or has been generated with unrelated objectives/indications in mind. The only exceptions are naturally occurring mouse mutants, such as leptin-deficient ob/ob and tubby mice, where the underlying mutations have been identified by positional cloning efforts [65,66].

Caenorhabditis elegans

The worm is not necessarily an organism that comes to mind when thinking about metabolic diseases, nevertheless, at the present time only the genome of *Caenorhabditis elegans* has been subjected to a nearly comprehensive screen specifically for a lipid storage phenotype. Many human disease-associated genes are present in the *C. elegans* genome and metabolic processes are evolutionary highly conserved, therefore it seems reasonable to use the worm for the identification of metabolic disease targets [67]. Ashrafi *et al.* [68] knocked down the function of 16,757 worm genes by applying RNAi covering nearly the complete genome while screening for lipid storage phenotypes. Although worms lack proper adipose tissue, they have specialized intestinal cells that store lipids very effectively. Considering that obesity and the metabolic syndrome are diseases characterized by improper storage of lipids, it is probably not too surprising that several genes with known metabolic functions were identified that validate the approach. Overall, the screen yielded 305 genes that caused a reduction in fat storage when inactivated and 112 genes that caused an increase in fat storage. Depending on the level of stringency applied, it is possible to assign human homologues to 30–50% of the identified worm genes. According to the authors, most of these genes have not been previously implicated in regulating fat storage and therefore could provide novel candidate targets for obesity and associated diseases.

Drosophila melanogaster

An initial analysis of the fruit fly genome for probable orthologues of human disease genes identified matches to 75% of the 1378 human disease loci, indicating that flies are more closely related to humans than worms. In particular, genes implicated in metabolic disorders are almost always conserved, with the insulin receptor signaling pathway being a prominent example [69]. Flies have abdominal adipose-like tissue, which is morphologically and physiologically similar to mammalian adipose tissue. Doane [70,71] even identified a naturally occurring mutant fly line with an obesity-related phenotype arising from a mutation in a protein of unknown function, which combines WD40 and tetratricopeptide protein–protein interaction motifs [72]. Furthermore, it has been reported that Drosophila has specialized neuronal cells that produce insulin and are functionally orthologous to mammalian β-cells. Ablation of these insulin-producing cells in flies generates phenotypes similar to diabetes [73].

The basic metabolic processes regulating fat as well as carbohydrate metabolism in Drosophila are conserved and the tools for genetic screens are well established, therefore, researchers at DeveloGen (http://www.develogen.com) set out to screen the Drosophila genome for metabolic phenotypes. A screen for modifiers of UCP activity yielded genes involved in the regulation of UCP activity, exemplified by an inner mitochondrial transmembrane protein, which suppresses UCP activity in flies as well as in mammals [74]. A second screen determining the overall triglyceride content of >10,000 mutant Drosophila lines, demonstrates that it is possible to detect fly lines with either significantly increased triglyceride levels (obese), or significantly decreased triglyceride levels (lean; Figure 1). The fact that the triglyceride levels of the majority of fly lines is within 30% of the average strongly suggests that mutations in most genes have only a limited effect on overall triglyceride storage, whereas mutations in relatively few genes have a pronounced effect on triglyceride storage. Although this point might seem trivial, it nicely illustrates the power of functional screens in model organisms, where animals can be kept under standardized conditions to produce metabolic phenotypes based on mutations in genes that probably could not have been predicted. That predictions about gene functions are inherently difficult even for well characterized genes was demonstrated once again by example of HSL, which until recently was believed to be the rate-limiting

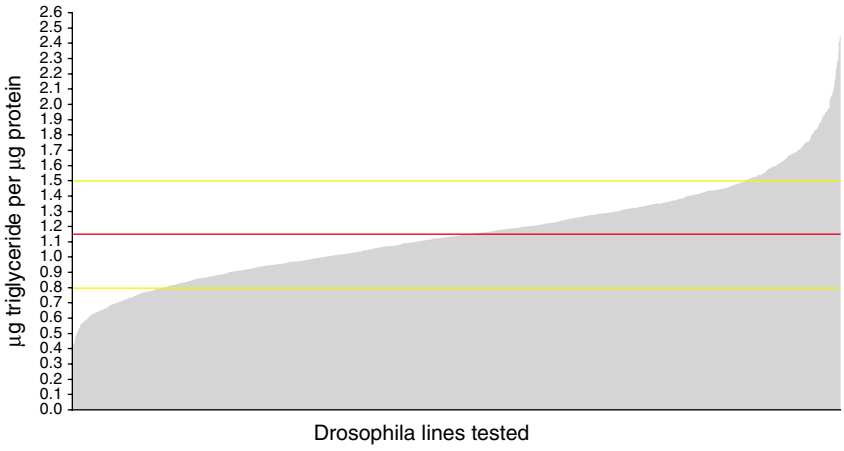

Figure 1. Overall triglyceride levels in mutant Drosophila lines were determined in relationship to protein content. The distribution of triglyceride levels reveals that most flies carrying a *P* element-induced mutation in one particular gene have an average triglyceride content of around 1.1 µg triglyceride per µg protein (red line). Wildtype flies vary in their triglyceride content by about 30% (yellow lines), suggesting that variations beyond this threshold are significant. Displayed are triglyceride levels of a batch of 2108 fly lines analyzed under identical conditions.

enzyme for adipocyte lipolysis but turned out to be less than critical for triglyceride hydrolysis in HSL-deficient mice [75,76].

Many of the genes identified in these Drosophila screens are not only highly relevant to energy metabolism in flies but are clearly involved in mammalian metabolic processes, such as adipogenesis, fat or carbohydrate metabolism, and insulin receptor signalling. In addition, many genes that had not been previously implicated in the regulation of energy metabolism have also been identified and therefore represent novel candidate target genes.

The potential of model organisms

Compared to expression profiling or human genome scans, functional screens in model organisms have several advantages when it comes to target discovery in metabolic diseases: (1) Metabolic diseases are of multi-factorial origin, caused by a complex interplay of the genetic predisposition of an individual and environmental factors; dissecting cause and consequence is only possible if all variables except for one are kept constant, which is usually not feasible in approaches involving human subjects. (2) Key metabolic processes, including appetite, fat and carbohydrate metabolism, as well as basic metabolic rate are governed by complex interwoven pathways that are characterized by considerable redundancy and a variety of feedback mechanisms: identification of genes that are either rate-limiting and/or lack redundancy is most easily accomplished in whole-organism screens. (3) Drugs for metabolic diseases usually need to be administered chronically and therefore require excellent safety profiles, essentially devoid of target-related adverse effects; screening in model organism quickly reveals if inhibition of a certain gene function has the potential to generate unwanted adverse effects. Thus, functional screens for metabolic disease targets in model organisms still present an enormous and so far largely untapped potential for future target discovery efforts in metabolic diseases.

Conclusion

Target discovery in the area of metabolic diseases is a field of intense investigation, and will be for years to come, because of the obvious ethical and financial challenges for human health and healthcare systems posed by metabolic diseases. Although highly interesting targets are currently pursued in drug discovery efforts, the pathophysiological basis of metabolic diseases is only poorly understood. Identification of targets associated with the early pathophysiology of metabolic diseases could lead to the development of preventative therapeutic strategies suitable for earlier intervention than is possible with current treatments. Large-scale expression profiling, the search for human susceptibility genes, as well as model organism screens are highly complementary approaches for target discovery in metabolic disease. The combination of these genomic approaches will deliver novel insights regarding the etiology of T2D, obesity and metabolic syndrome and thus undoubtedly lead to the development of novel and improved therapies based on highly validated drug targets.

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References

1 Ogden, C.L. *et al.* (2003) Epidemiologic trends in overweight and obesity. *Endocrinol. Metab. Clin. North Am.* 32, 741–760
2 Narayan, K.M. *et al.* (2003) Lifetime risk for diabetes mellitus in the United States. *J. Am. Med. Assoc.* 290, 1884–1890
3 Ford, E.S. *et al.* (2002) Prevalence of the metabolic syndrome among US adults. *J. Am. Med. Assoc.* 287, 356–359
4 Mokdad, A.H. *et al.* (2003) Prevalence of obesity, diabetes and obesity-related health risk factors, 2001. *J. Am. Med. Assoc.* 298, 76–79
5 Malik, T. and Trence, D.L. (2003) Treating diabetes using oral agents. *Prim. Care* 30, 527–541

6 Bays, H. and Dujovne, C. (2002) Pharmacotherapy of obesity: currently marketed and upcoming agents. *Am. J Cardiovasc. Drugs* 2, 245–253
7 Bernal-Mizrachi, E. *et al.* (2003) Gene expression profiling in islet biology and diabetes research. *Diabetes Metab. Res. Rev.* 19, 32–42
8 Gabrielsson, B.L. *et al.* (2000) Partial Genome scale analysis of gene expression in human adipose tissue using DNA array. *Obes. Res.* 8, 374–384
9 Sanchez, J.C. *et al.* (2001) The mouse SWISS-2D PAGE database; a tool for proteomics study of diabetes and obesity. *Proteomics* 1, 136–163
10 Atzmon, G. *et al.* (2002) Differential gene expression between visceral and subcutaneous fat depots. *Horm. Metab. Res.* 34, 622–628
11 Yang, Y-S. *et al.* (2002) The gene expression profiling of human visceral adipose tissue and ist secretory functions. *Biochem. Biophys. Res. Commun.* 300, 839–846
12 Gabrielsson, B.G. *et al.* (2003) High expression of complement components in omental adipose tissue in obese men. *Obes. Res.* 11, 699–708
13 Linder, K. *et al.* (2004) Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. *J. Lipid Res.* 45, 148–154
14 Klaus, S. (2004) Adipose tissue as a regulator of energy balance. *Curr. Drug Targets* 5, 241–245
15 Li, J. *et al.* (2002) Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. *Am. J. Physiol. Endocrinol. Metab.* 282, 1334–1341
16 Moraes, R.C. *et al.* (2003) Study of the alteration of gene expression in adipose tissue of diet-induced obese mice by microarray and reverse transcription-polymerase chain reaction analyses. *Endocrinology* 144, 4773–4782
17 Nadler, S.T. *et al.* (2000) The expression of adipogenic genes is decreased in obesity and diabetes mellitus. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11371–11376
18 Soukas, A. *et al.* (2000) Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev.* 14, 963–980
19 Gómez-Ambrosi, J. *et al.* (2004) Gene expression profile of omental adipose tissue in human obesity. *FASEB J.* 18, 215–217
20 Yang, X. *et al.* (2004) Evidence of impaired adipogenesis in insulin resistance. *Biochem. Biophys. Res. Commun.* 317, 1045–1051
21 Weisberg, S.P. *et al.* (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808
22Xu, H. *et al.* (2003) Chronic inflammation in fat plays a crucial role in the development ob obesity-related insulin resistance. *J. Clin. Invest.* 112, 1821–1830
23 Lehrke, M. and Lazar, M.A. (2004) Inflamed about obesity. *Nat. Med.* 10, 126–127
24 Green, H. and Kehinde, O. (1975) An established preadipose cell line and ist differentiation in culture. II. Factors affecting the adipose conversion. *Cell* 5, 19–27
25 Guo, X. and Liao, K. (2000) Analysis of gene expression profile during 3T3-L1 preadipocyte differentiation. *Gene* 251, 34–53
26 Jessen, B.A. and Stevens, G.J. (2002) Expression profiling during adipocyte differentiation of 3T3-L1 fibroblasts. *Gene* 299, 95–100
27 Burton, G.R. *et al.* (2002) Microarray analysis of gene expression during early adipocyte differentiation. *Gene* 293, 21–31
28 Soukas, A. *et al.* (2001) Distinct transcriptional profiles of adipogenesis *in vivo* and *in Vitro*. *J. Biol. Chem.* 276, 34167–34174
29 Urs, S. *et al.* (2004) Gene expression profiling in human preadipocytes and adipocytes by microarray analysis. *J. Nutr.* 134, 762–770
30 Lowell, B.B. and Spiegelman, B.M. (2000) Towards a molecular understanding of adaptive thermogenesis. *Nature* 404, 652–660
31 Unami, A. *et al.* (2004) Comparison of gene expression profiles between white and brown adipose tissues of rat by microarray analysis. *Biochem. Pharmacol.* 67, 555–564
32 O'Brien, R.M. and Granner, D.K. (1996) Regulation of gene expression by insulin. *Physiol. Rev.* 76, 1109–1161
33 Yechoor, V.K. *et al.* (2002) Coordinated patterns of gene expression for substrate and energy metabolism in skeletal muscle of diabetic mice. *Proc. Natl. Acad. Sci. U. S. A.* 16, 10587–10592
34 Rome, S. *et al.* (2003) Microarray profiling of human skeletal muscle reveals that insulin regulates appr. 800 genes during a hyperinsulinemic clamp. *J. Biol. Chem.* 278, 18063–18068
35 Sreekumar, R. *et al.* (2002) Gene expression profile in skeletal muscle of type-2diabetes and the effect of insulin treatment. *Diabetes* 51, 1913–1920
36 Patti, M.E. *et al.* (2003) Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8466–8471
37 Yang, X. *et al.* (2002) Microarray profiling of skeletal muscle tissues from equally obese, non-diabetic insulin-sensitive and insulin-resistant Pima Indians. *Diabetologia* 45, 1584–1593
38 Lee, Y.H. *et al.* (2003) Identification of differentially expressed genes in skeletal muscle of non-diabetic insulin-resistant and insulin-sensitive Pima Indians by differntial display PCR. *Diabetologia* 46, 1567–1575
39 Link, J.T. (2003) Pharmacological regulation of hepatic glucose production. *Curr. Opin. Investig. Drugs* 4, 421–429
40 Ferrante, A.W. *et al.* (2001) Effects of leptin deficiency and short-term repletion on hepatic gene expression in genetically obese mice. *Diabetes* 50, 2268–2278
41 Dhahbi, J.M. *et al.* (2003) Hepatic gene expression profiling of streptozotocin-induced diabetes. *Diabetes Technol. Ther.* 5, 411–420
42 Lan, H. *et al.* (2003) Gene expression profiles of nodiabetic and diabetic obese mice suggest a role of hepatic lipogenic capacity in diabetes susceptibility. *Diabetes* 52, 688–700
43 Dor, Y. *et al.* (2004) Adult pancreatic beta cells are formed by self-duplication rather than stem cell differentiation. *Nature* 429, 41–46
44 Sturis, J. *et al.* (2003) GLP-1 derivative liraglutide in rats with beta-cell deficiencies: influence of metabolic state on beta cell mass dynamics. *Br. J. Pharmacol.* 140, 123–132
45 Ahren, B. *et al.* (2002) Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4 week study period in type II diabetes. *Diabetes Care* 25, 869–875
46 Conarello, S.L. (2003) Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6825–6830
47 Popisilik, J.A. *et al.* (2003) Dipeptidyl peptidase IV inhibitor treatment stimulates beta cell survival and islet neogensis in streptozotocin-induced diabetic rats. *Diabetes* 52, 741–750
48 Shalev, A. *et al.* (2002) Oligonucleotide microarray analysis of intact human pancreatic islets: Identification of glucose-responsive genes and a highly regulated TGFβ signaling pathway. *Endocrinology* 143, 3695–3698
49 Li, M. *et al.* (2003) Analysis of expression profiles of islet-associated transcription and growth factors during b-cell neogenesis from duct cells in partially duct-ligated mice. *Pancreas* 27, 345–355
50 Albrektsen, T. *et al.* (2002) Novel genes regulated by the insulin sensitizer rosiglitazone during adipocyte differentiation. *Diabetes* 51, 1042–1051
51 Okuno, M. *et al.* (2002) Isolation of up- or down-regulated genes in PPAR gamma-expressing NIH-3T3 cells during differentiation into adipocytes. *FEBS Lett.* 519, 108–112
52 Rothberg, B.E. *et al.* (2001) The characterization of PPAR alpha ligand drug action in an *in vivo* model by comprehensive differential gene expression profiling. *Funct. Integr. Genomics* 1, 294–304
53 Edvardsson, U. *et al.* (2003) Hepatic protein expression of lean mice and obese diabetic mice treated with peroxisome proliferator-activated receptor activators. *Proteomics* 3, 468–478
54 McCarthy, M.I. (2003) Growing evidence of diabetes susceptibility genes from genome scan data. *Curr. Diab. Rep.* 3, 159–167
55 Snyder, E.E. *et al.* (2004) The human obesity gene map: The 2003 update. *Obes. Res.* 12, 369–439

56 Loos, R.J.F. *et al.* (2003) Genome-wide linkage scan for the metabolic syndrome in the HERITAGE family study. *J. Clin. Endocrinol. Metab.* 88, 5935–5943
57 Liu, Y-J. *et al.* (2003) Molecular and genetic mechanisms of obesity: implications for future management. *Curr. Mol. Med.* 3, 325–340
58 Walder, K. *et al.* (2003) Obesity and diabetes gene discovery approaches. *Curr. Pharm. Des.* 9, 1357–1372
59 McCarty, M.I. (2004) Progress in defining the molecular basis of type 2 diabetes mellitus through susceptibility-gene identification. *Hum. Mol. Genet.* 13, R33–R41
60 Nusslein-Volhard, C. and Wieschaus, E. (1980) Mutations affecting segment number and polarity in drosophila. *Nature* 287, 795–801
61 Shin, J.T. and Fishman, M.C. (2002) From zebrafish to human: modular medical models. *Annu. Rev. Genomics Hum. Genet.* 3, 311–340
62 Brown, S.D.M. and Balling, R. (2001) Systematic approaches to mouse mutagenesis. *Curr. Opin. Genet. Dev.* 11, 268–273
63 Zambrowicz, B.P. and Sands, A.T. (2003) Knockouts model the 100 best-selling drugs – will they model the next 100? *Nat. Rev. Drug Discov.* 2, 38–51
64 Zambrowicz, B.P. *et al.* (2003) Predicting drug efficacy: knockouts model pipeline drugs of the pharmaceutical industry. *Curr. Opin. Pharmacol.* 3, 563–570
65 Zhang, Y. *et al.* (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* 372, 425–432
66 Kleyn, P. *et al.* (1996) Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family. *Cell* 85, 281–290
67 Aboobaker, A.A. and Blaxter, M.L. (2000) Medial significance of *Caenorhabditis elegans*. *The Finnish Medical Society Duodecim. Ann. Med.* 32, 23–30
68 Ashrafi, K. *et al.* (2003) Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature* 421, 268–272
69 Lasko, P. (2002) Diabetic flies? Using *Drosophila melanogaster* to understand the causes of monogenic and genetically complex diseases. *Clin. Genet.* 62, 358–367
70 Doane, W.W. (1960) Developmental physiology of the mutant female sterile (2) adipose of *Drosophila melanogaster*. I. Adult morphology, longevity, egg production, and egg lethality. *J. Exp. Zool. Oct.* 145, 1–21
71 Doane, W.W. (1960) Developmental physiology of the mutant female sterile (2) adipose of *Drosophila melanogaster*. II. Effects of altered environment and residual genome on its expression. *J. Exp. Zool. Oct.* 145, 23–41
72 Häder, T. *et al.* (2003) Control of triglyceride storage by a WD40/TPR-domain protein. *EMBO Rep.* 4, 511–516
73 Rulifson, E.J. *et al.* (2002) Ablation of insulin-producing neurons in flies: Growth and diabetic phenotypes. *Science* 296, 1118–1120
74 Schreiter, K. *et al.* (2004) DG175 – An inner mitrochondrial transmembrane protein identified in a UCP – modifier screen in Drosophila. *Obes. Res.* 12, 170–172
75 Osuga, J. *et al.* (2000) Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. *Proc. Natl. Acad. Sci. U. S. A.* 97, 787–792
76 Wang, S.P. (2001) The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. *Obes. Res.* 9, 119–128