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Machine learning based bioinformatics analysis of intron usage alterations and metabolic regulation in adipose browning

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Abstract: Adipose tissue is the major energy depot of the body and is considered an endocrine organ. Adipose tissue involves many different cell types, first and foremost, the adipocytes. White adipose cells that store fat and brown adipocytes that take part in lipid oxidation and heat generation are the most common cell types in adipose tissue. Even though brown adipocytes which have a high number of mitochondria and high fat-burning capacity are rare in adults, they are abundant in newborns and rodents. White adipocytes can gain a temporal brown-like character with a process called browning, which can be induced with cold exposure providing white adipocytes with increased fat-burning capacity. Adipose tissue is the main tissue associated with obesity; therefore, the browning process has the potential to be used in the treatment of obesity. Here, we made use of machine learning techniques to better understand the browning mechanisms. We applied a computational approach based on generalized linear models (GLM) and decision trees for the identification and classification of alternative splicing events, followed by downstream bioinformatics analysis for the detection of differential regulatory events in the transcriptome of the adipocyte browning. Our analyses identified possible extracellular alterations in response to changes in cellular shape via alternative splicing events and remarkably an intron retention event on the Upstream stimulatory factor2 (Usf2) gene that may alter the activity of the regulator and take part in the regulation of the browning process. Targeted therapies for induction of the browning process via regulation of Usf2 may prevent and treat obesity which is a widespread health condition. To the best of our knowledge, this is the first study that combines alternative splicing events with regulatory network inference to reveal the mechanism of the browning process. Our methodology has the potential to reveal many other disease-related mechanisms and lead to novel therapy strategies.

Key words: Adipocytes, browning, obesity, alternative splicing, GLM, machine learning

1. Introduction

The use of machine learning and deep learning models in life sciences allowed researchers and clinicians to extract novel information from large datasets and build prediction tools. Modeling and interpretation of signals and images from medical devices [1–3], use of patient profiles for diagnosis and subtyping of diseases [4–6], designing medical human-computer interaction devices [7], keeping track of crop growth [8, 9] are made possible by the use of such models. Transcriptome, which provides a complete RNA landscape of the samples, are often used to identify and classify diseases [10, 11] as well as more recently being used for investigating mechanisms of biological processes. In our case, brown, white, and beige adipocytes have different transcriptomic landscapes leading to the metabolic differences between them allowing us to investigate the

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browning process. We hypothesize that transcriptome-based studies combined with advanced computational analysis utilizing modeling via deep/machine learning techniques have the potential to identify the gene level alterations, regulators, and potential drug targets to induce browning in obese patients.

In recent years, obesity has become a major health condition with a rapidly increasing number of patients around the globe. In 1997, obesity was recognized as an epidemic by the World Health Organization (WHO), in 2022, the WHO reported that overweight and obesity affect almost 60% of adults and nearly 30% of children in the WHO European Region. Diagnosis of obesity is commonly based on body mass index (BMI) being larger than 30 [12], although recently automated diagnosis tools based on modeling the clinical data using machine learning methods have been proposed [13]. Abnormal or excessive fat accumulation in obesity presents a risk to health as the high amount of body fat is highly correlated with other metabolic diseases such as type II diabetes [14]. Currently, obesity treatments are limited to strict diet plans, exercise programs, or surgery. There is a growing need for new therapeutic strategies to prevent or treat obesity.

Obesity arises in adipose tissue which is the main energy depot of the body. Adipose tissue is considered an endocrine organ and involves many different cell types such as blood cells, fibroblasts, and nervous cells but most importantly, it involves two distinct types of adipocytes, white and brown. White adipose tissue stores fat as an energy source for the organism while brown adipocytes have two main functions, lipid oxidation and heat generation [15]. Our understanding of their origins has changed significantly in the previous years, it is shown that these two cell types originate from different types of mesenchymal cells. Recently it is reported that brown adipocytes (BATs) arise from Myf5-expressing myogenic precursor cells while white adipocytes (WATs) arise from Myf5-negative precursors [16]. Also, adipocytes differentiate from preadipocyte cells with a process called adipogenesis.

Brown adipocytes are common in newborns, rodents, and hibernating animals but in recent years, traces of brown adipose tissue were found in adult humans, and with their fat-burning characteristics, they have promising potential for the treatment of obesity. In addition, white adipocytes can gain a temporal brown-ish character with cold or chemical exposure [17]. This biological process is called browning and these cells are named beige, bright or brown-in-white adipocytes. Beige adipocytes generate heat with the increasing activity of Uncoupling Protein 1 (Ucp-1) [18]. The browning process is regulated by multiple factors and signaling pathways. PGC-1α protein is a master regulator of mitochondrial biogenesis and oxidative metabolism in adipocytes and can induce the expression of Ucp1. Also, fibroblast growth factor 21 (FGF21) acts as an autocrine factor in White Adipose Tissue (WAT) browning. Bone morphogenetic proteins (BMPs), especially BMP4 and BMP7 induce the browning of WAT and increase the metabolic rate of the body. Blocking of TGF-β/Smad3 signaling protects mice from obesity by promoting the browning process. RGC-32) deficiency increases WAT browning and body metabolic rate [14, 17–19]. The browning process can be induced via long-term cold exposure, overexpression of a subset of miRNAs, genes, and chemicals such as rapamycin, butene, and acetate. These chemicals are known as "browning agents". However, the best-known browning agent is cold exposure [20].

Alternative splicing (AS) is a mechanism of regulation of gene expression that allows the generation of more than one mRNA species from a gene, AS alters the combinations of exons to be translated into the protein, hence multiple proteins can be coded by a single gene, greatly increasing the biodiversity [21]. With the alternative splicing mechanism, mRNAs that differ in untranslated regions (UTR) or coding sequences can be generated. It is reported that nearly all eukaryotic organisms have pre-mRNA splicing [22]. Since about

95% of human genes are multi-exonic and undergo a splicing event, most of the mRNAs are variably expressed between cells and tissues. Like many other biological processes, any disruption in AS machinery may result in a perturbed biosystem and cause diseases. 60% of pathogenic mutations affect splicing regions rather than protein-coding regions [23].

Detection of alternative splicing of events is based on mapping of transcript reads on the reference genome and counting the reads that originated from alternatively spliced forms of genes. Alternatively, de novo assembly of transcripts may be applied if a reliable reference genome is not available. Machine learning and deep learning models are required for accurate identification of such events as preset thresholds or rules for identification or classification cannot be applied to transcriptome data with high levels of noise and sample-to-sample variation. Deep learning methods are suitable for datasets without a reference genome however they require large sets of data for accurate predictions [24]. Machine learning-based methods which can perform adequately on smaller sets of data, hence they are preferred for well-studied organisms such as humans, for which an established reference genome is available. Prediction tools based on a variety of algorithms have been developed recently to identify and classify alternative splicing events. One such machine learning-based tool is ASpli [25], which uses GLMs for identification of the aberrant expression events and builds a sample-specific rule-based decision tree for the classification of the events. ASpli estimates the differential expression signals via GLMs after modeling the counts assuming a negative binomial distribution. A number of metrics such as Percent Spliced-In (PSI) and the Percent of Intron Retention (PIR) are used to quantify the relative weight of inclusion evidence for aberrant expression events. The differential expression signals are then used to identify and classify the ES, IR, Alt3', Alt5', and novel splicing events in the sample, the events are reported together with the confidence level of identification.

The performance of ASpli was evaluated using tumor transcriptome data and simulated alternative splicing events and compared to other tools available in the literature, leafcutter [26], MAJIQ [27] and rMATS [28]. ASpli outperformed the three tools by achieving higher sensitivity and specificity. The sensitivity and specificity of the tools varied between 0.87-0.91 and 0.08-0.18 respectively, while the sensitivity of ASpli was 0.96-0.99 and specificity was 0.23-0.40 for the datasets analyzed. Currently, 51 alternative splicing tools are listed in Bioconductor BiocView, and downloads of ASPli ranks at the top 5 among the tools devoted only to alternative splicing prediction and analysis (retrieved via BiocView) [29].

Identifying the splicing events and how they regulate the disease mechanisms is crucial and potentially has therapeutic uses, however, genome-wide alternative splicing events in the browning process have not been reported in the literature. Here we aim to reveal the alternative splicing events in the browning process, which may take part in the regulation of the process. We used publicly available RNA-Seq data [30] of white and beige adipocytes collected at different temperatures to analyze the differential expression, used the Reporter Regulatory Elements Analysis (RREA) algorithm on the transcription factor-gene network and analyzed the alternative splicing events using the ASpli package. Our analyses indicated that transcription factor Usf2 potentially undergoes an alternative splicing event (intron retention) and this change affects its function as a regulator. Also, we identified 29 post-transcriptionally modified regulators in reporter regulatory elements analysis (RREA) and 487 statistically significant alternative splicing events. Hence we have shown that by using machine learning and combining alternative splicing events with RREA, clinically relevant targets that regulate the process of adipose browning can be identified.

2. Materials and methods

A tutorial and script of the methods described below are given as an R markdown notebook and also in pdf format at: https://github.com/hkarakurt8742/adipose_browning

2.1. Transcriptome data and preprocessing

The RNA-Seq data of cold beige, warm beige, and warm white cells from the data set GSE108077 [30] was used in this study. The study is conducted using mice growth at a constant 23 °C with a 12h light/12h dark cycle. Mice were exposed to cold (4°C for 1 week (cold brown/beige adipocyte samples), and subsequently incubated at 30 °C for 4 weeks (warm brown/beige adipocyte samples). Mice born and maintained at 30 °C at all times were used for warm white adipocyte samples. The selected 12 samples, RNA-Seq data of warm white (30 °C), warm brown (30 °C), and cold beige (4°C) cells are used for our analyses. Previously, researchers analyzed the same data set using different preprocessing tools and investigated differential expression along with ChIP-Seq data of the same samples and identified chromatin-based cellular reprogramming in beige adipocytes with temperature, but not in brown adipocytes. Different than our analyses, the main article used RNA-Seq samples to investigate only the gene expression but not the alternative splicing events and their regulatory effects.

Raw reads were downloaded from NCBI SRA [31] using the SRA Toolkit. Reads were aligned to the mouse genome (Ensembl GRCm39) using the STAR aligner [32]. SAM Files are converted to BAM files, sorted, and indexed using Samtools [33].

2.2. Alternative splicing and differential expression analyses

Reads in BAM files are counted using the Ensembl Mouse Genome (GRCm39) genome annotation file [34] using the ASPli package in R [35]. For differential expression analysis, gene counts are extracted from the ASPli object and analyzed via DESeq2 [36] package. Genes with a total count lower than 36 are removed (Average 3 counts for each sample). Alternative splicing analyses, GLM based event detection, event assignment using a set of decision criteria are done in the ASPli package. The conversion table of Ensembl gene IDs to Gene Symbols was downloaded using the Biomart package [37] and converted using the vlookup function in the expss package. Enrichment analyses were applied to gene sets using clusterProfiler [38] package for enrichment in Gene Ontology Biological Process, Cellular Component and Molecular Function terms [39]. Differential gene analysis was done between cells exposed to different temperatures.

2.3. Analyses of exon and intron counts

ASPli package is used for the identification and classification of ES, IR, Alt3', Alt5', and novel splicing events, the count matrices for exons and introns are also extracted for further regulatory analysis. Features with total counts lower than 36 (Average of 3 counts for each sample) were removed in further analyses.

2.4. Reporter regulatory elements analysis

Reporter metabolite [40] and reporter pathway [41] algorithms are often used to identify metabolic alterations and calculate p-values for metabolites and pathways using the differential expression results throughout a sub-network. Here, we applied the same algorithm to calculate p-values for transcription factors through p-values of nonregulatory genes. The gene-transcription factor for the mouse was downloaded from RegNetwork [42]

and analyses were done in Matlab 2020 using in-house scripts. Construction and applications on networks were done in Cytoscape 3.9 [43].

3. Results

3.1. Differential expression analysis results

Differential expression analysis identified 3983 differentially expressed genes (Adjusted p-Value < 0.01 and absolute Log2FoldChange > 1.5) (Supplementary Excel Sheet Table 1) as a response to heat, among 22706 genes. Enrichment analysis indicated that these genes are highly associated with energy metabolism, metabolic processes, mitochondrial membrane, and respiratory complexes (Supplementary Figures 1-2). As expected, pathways associated with energy metabolisms such as the ATP metabolic process, oxidative phosphorylation, and fatty acid metabolic processes were downregulated with heat. Cellular component terms indicated that extracellular matrix organization and collagen trimers were upregulated with heat while terms associated with mitochondria and organelle membranes were downregulated with heat. This result indicates that an increase in heat modifies the extracellular matrix regulation, however, the organelle membrane is affected oppositely.

3.2. Alternative splicing analysis results

ASPli package uses an implementation of edgeR for differential feature testing. GLM-based analyses identified 487 statistically significant alternative splicing (AS) events (Adjusted p-value < 0.05) (Supplementary Excel Sheet Table 2) and assigned these to 4 main types of alternative splicing events; exon skipping (ES), intron retention (IR), alternative 3' splice site usage (Alt3ss) and alternative 5' splice site usage (Alt5ss) using a set of rules after deriving the criteria from the data model. The distribution of AS events is shown in Supplementary Figure 3, Venn diagram of alternative splicing events is shown in Supplementary Figure 4.

Enrichment analysis was applied to the genes that undergo each type of alternative splicing event. Genes with Alt3ss and Alt5ss events were not enriched in any GO terms. Genes with upregulated exon skipping (ES) events in response to heat were enriched in cellular respiration, TCA cycle, diphosphatases, and components of organelle membranes including Golgi and mitochondria while genes with downregulated exon skipping events were enriched in cell cortex, actin filament bundle, and actin-binding (Supplementary Figures 5-6).

Similarly, genes with significantly upregulated intron retention (IR) events are enriched in metabolic processes and organelle inner membranes but interestingly genes with downregulated IR events are enriched in the extracellular matrix and collagen trimers (Figure 1). It is possible that these genes undergo alternative splicing events to regulate the cell and organelle structure, more commonly, extracellular matrix, as opposed to genes with significant ES events being involved in pathways associated with cell cortex and diphosphatase activities. Analyses indicate that IR events are as important and effective as ES (and differential expression) events in the alteration of extracellular matrix organization and collagen trimers in the browning process. Change of morphology in adipocytes due to loss or gain of lipid storage possibly triggers the extracellular regulation directly [44, 45]. Count matrices for exons and introns were extracted from the ASPli object for further analysis. The log2 counts of exons and introns with ES and IR events are shown in Figure 2 as a heatmap. The number of plotted introns is limited to 56 (the same number of plotted exons) for better visualization. A complete table of introns and exons is given in (Supplementary Excel Sheet Table 3-4).

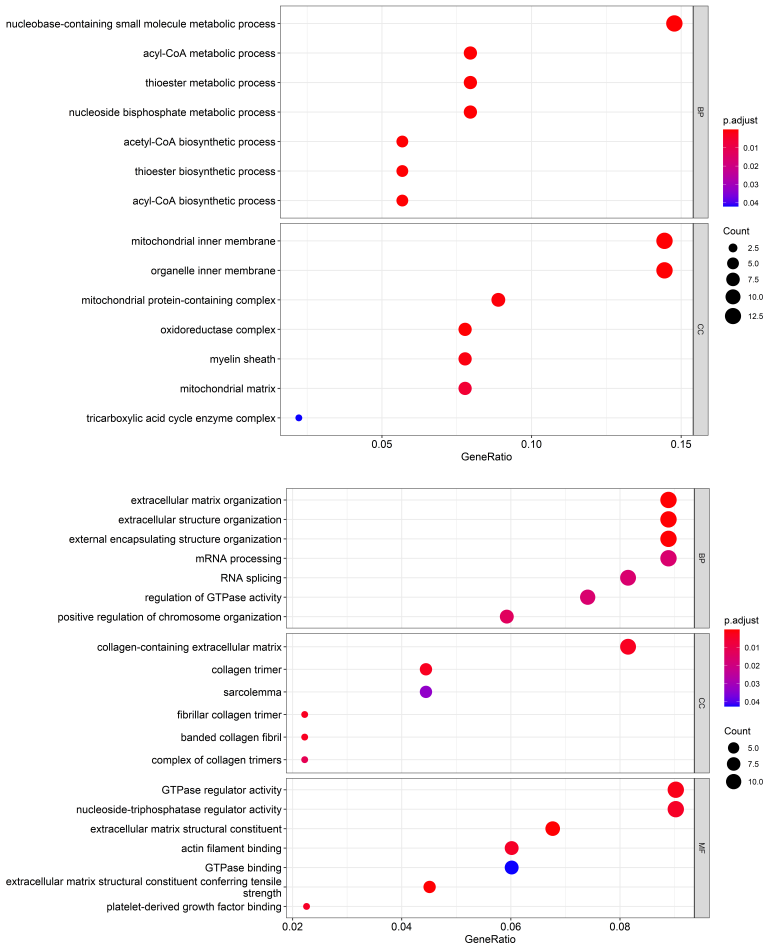

Figure 1. Analysis results of genes with significant intron retention events (Top: Upregulated, Bottom: Downregulated).

3.3. Reporter regulatory elements analysis (RREA) results

As mentioned above, reporter feature algorithms are mainly used for metabolic networks, but they can be applied to any biological network. We propose the use of reporter features algorithm for the transcription factor-gene network to reveal its potential to identify alterations in transcription factors based on their activity/function rather than the mRNA level. Since all transcription factors in the network were subject to differential expression analysis themselves, but RREA calculates the statistics based only on their target genes, it is hypothesized that transcription factors with significant RREA results, but nonsignificant differential expression potentially undergo a process that alters the activity/function, potentially a posttranslational modification. In RREA, p-values of differential expression analysis (Section 3.1) of target genes were used to calculate a function/activity-based p-value value for transcription factors. Our analysis identified 63 significantly (Benjamini Hochberg Adj. p-value < 0.05) altered transcription factors among 1256 transcription factors (Supplementary Excel Sheet Table 5). Among these reporter regulators, 15 regulators are excluded from further analysis due to a low (4) or high (400) number of edges, since nodes with very high degrees may have roles in multiple processes while nodes

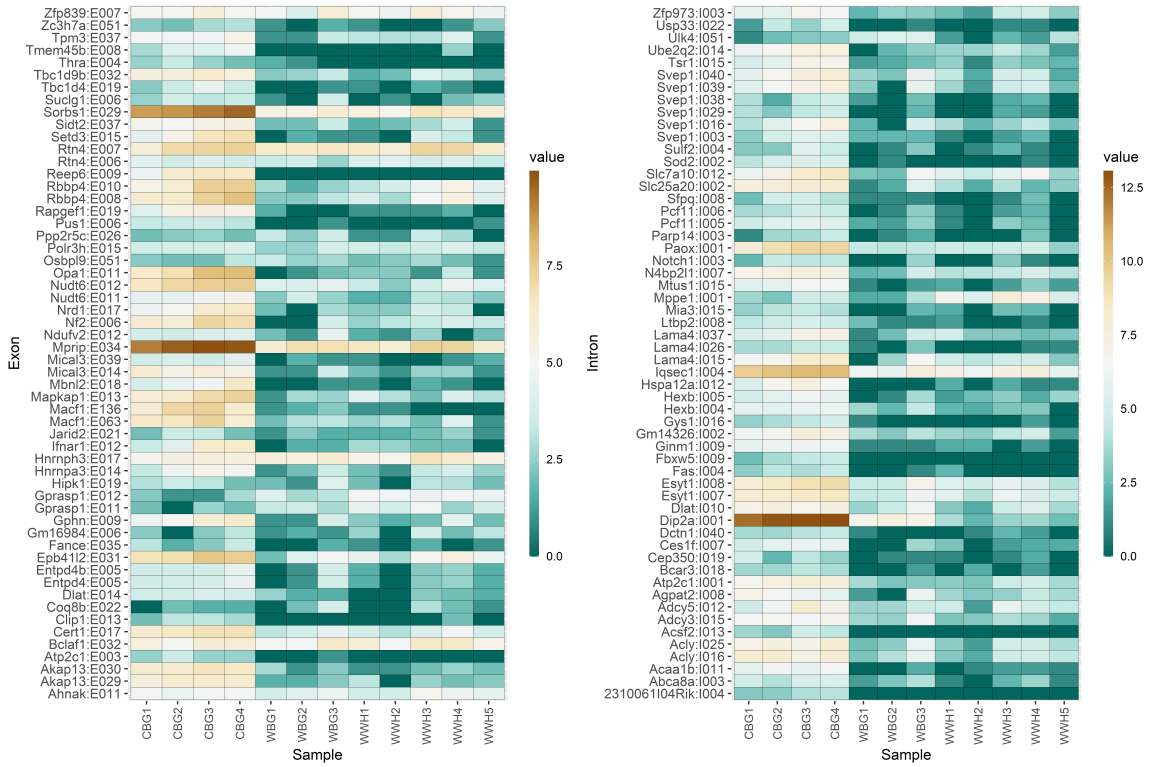

Figure 2. Heatmaps of alternatively spliced exons and introns (CBG cold beige, WBG warm beige, WWH warm white adipocytes).

with low degrees do not provide generalizable results. The remaining 48 transcription factors are considered reporter regulators (Supplementary File), these regulators are highly associated with insulin resistance and thyroid hormone signaling pathways (KEGG Database used for enrichment analysis) (Figure 3, Supplementary Figure 7) which are directly associated with energy metabolism. The thyroid hormone and thyroid signaling pathway are important regulators of glucose homeostasis and insulin resistance [46–48]. Also, previous studies indicated a connection between energy metabolism and Notch signaling [49], and inhibition of Notch signaling induces browning and improves insulin sensitivity [50]. PPAR signaling also takes part in the regulation of energy metabolism and PPAR is the master regulator of adipogenesis. PPAR activation in adipocytes induces the expression of genes involved in insulin resistance [51]. RREA reveals the significance of regulators based on the regulatory activity/function they exert on the expression of their target genes. Combining the gene expression changes in TFs and RREA results provides us with a broader perspective on gene regulation. If a transcription factor is not differentially expressed but is significantly altered in RREA, we hypothesize that these proteins might have gone alterations after transcription, such as a post-transcriptional modification. Twenty-nine transcription factors were identified as potentially posttranslationally modified regulators. Like reporter regulators, these regulators are highly associated with thyroid hormone signaling. However, the alterations in the regulation of PPAR signaling may occur on the transcription level while Thyroid signaling and Notch signaling may also be regulated post-transcriptionally (Figure 3).

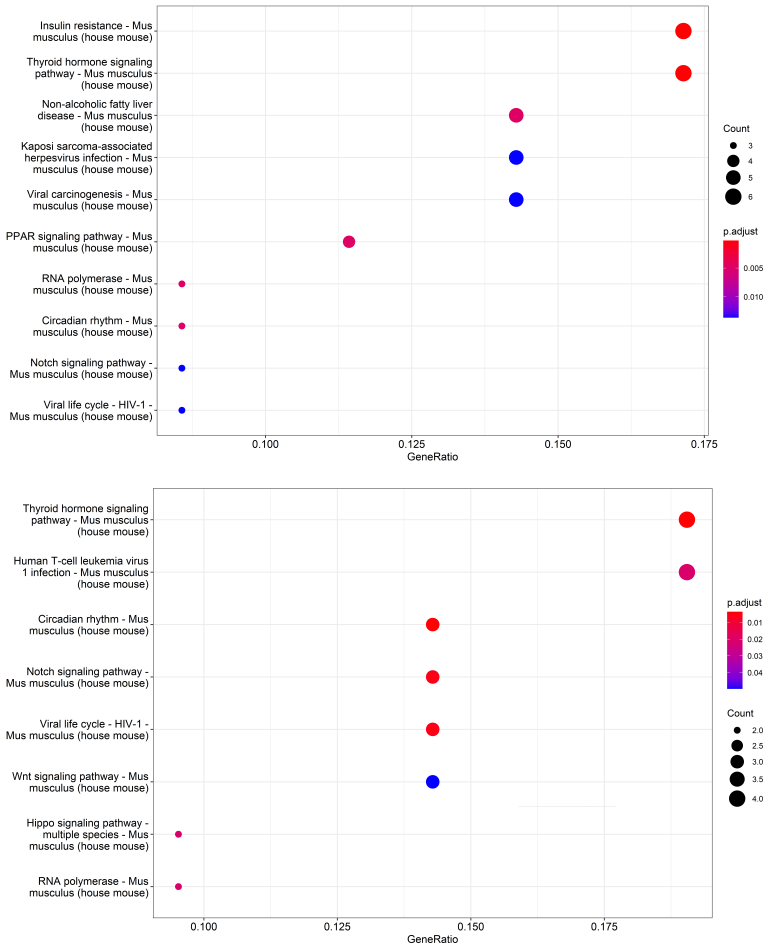

Figure 3. KEGG pathways enrichment analysis results of reporter regulators (a) Regulators with potential posttranscriptional modification (b)).

3.4. Integration of reporter regulators and alternative splicing events

Twenty-nine regulators were identified as posttranslationally modified regulator candidates, and the alternative splicing events in these regulators were also examined. Among these candidates, one regulator, Upstream Transcription Factor 2, Usf2 (ENSMUSG00000058239), is not differentially expressed itself but was found to be significantly altered in RREA (p-value = 0.026). Further, Usf2 is identified as a gene with significant intron retention events in our AS analysis (Figure 4).

Taken together, our findings indicated a possible alternative splicing event that affects the activity/function of this transcription factor which leads to differential expression of its target genes, although expression of Usf2 itself did not change. The network that shows the Usf2 and its differentially expressed target genes is given in Supplementary Figure 8 and Table 1 (Heatmap of expression levels of Usf2 regions are shown in Supplementary Figure 9). The Usf2-associated differentially expressed genes are found to be associated with muscle cell differentiation, mitochondrial genome maintenance, and energy metabolism including cellular respiration, ATP metabolic process (Supplementary Figure 10).

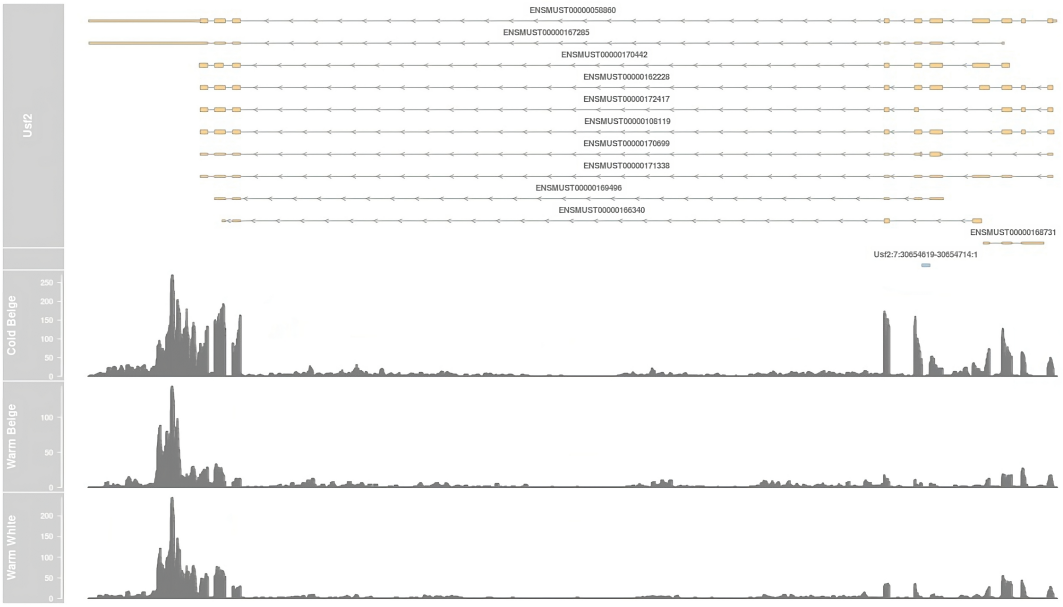

Figure 4. Alternative splicing events in Usf2.

4. Discussion and conclusions

The browning of white adipocytes is a reversible biological process, and it spontaneously occurs in cold temperatures to maintain the body temperature, utilization of this process in clinics holds the promise for the treatment of obesity and other metabolic disorders. We used machine learning-based detection and classification of differential alternative splicing events, intron, and exon usages and analyzed their connections with regulatory features. Interestingly, genes with significant IR events and variant introns that are expressed among cold beige, warm beige, and warm white adipocytes indicated a possible intron usage difference on genes that are associated with collagen trimer and extracellular matrix (ECM). Especially, the highest variant introns show remarkable association with organelle membrane and collagen trimer. Along with other ECM components, collagen is accumulated in the early stages of obesity as part of tissue remodeling. Here, we hypothesize an alteration in intron usage in cells with the browning process to regulate the ECM through possible alternative splicing events. Correlation analyses of exons and introns to the expression level of thermogenesis marker, Ucp1, did not identify any significant association of introns and exons to extracellular matrix and collagen but indicated a high association with energy metabolism as expected. Here, this result indicates that the main reason for the possible alternative splicing events is not caused by Ucp1 directly, and the regulators that act in this process must be investigated further.

The reporter regulatory features analyses (RREA), allow the identification of the main regulators in the process based on their activity, instead of their expression. Analyses identified 47 reporter regulators which are enriched in terms of insulin resistance, thyroid hormone signaling, and circadian rhythm. Regulators analyzed via RREA also have statistical analysis results based on their expression levels, these results were merged and compared. We hypothesized that regulators with nonsignificant expression changes but significant alterations in RREA may be subject to post-transcriptional modifications affecting their activity. In our analysis, 29 such regulators were found and examined in the context of alternative splicing events. These regulators are associated with thyroid hormone signaling and circadian rhythm.

Table 1. Summary of differentially expressed Usf2 targets.

Gene Symbol	Adjusted P-Value	Log2FoldChange	Summary
Slc2a5	4.83E-56	-7.74	Functions as a fructose transporter that has only low activity with other monosaccharides
Fasn	3.11E-27	-3.44	Multifunctional enzyme that catalyzes the de novo biosynthesis of long-chain saturated fatty acids starting from acetyl-CoA and malonyl-CoA in the presence of NADPH
Cox5b	2.69E-47	-2.90	Subunit of the cytochrome c oxidase complex and is known to be upregulated in brown adipocytes [55]
Speg	9.98E-10	-2.44	Regulates the growth and differentiation of arterial smooth muscle cells
Thrsp	1.16E-28	-2.40	Important in the synthesis of triglycerides and takes a role in the regulation of lipogenesis
Prkaca	9.82E-11	-2.16	Associated with numerous catabolic pathways including TCA [56]. It is suppressed by HIF-alpha, which is a target for the Mitf transcription factor [57]
Atp2a2	1.34E-60	-1.69	Catalyzes the hydrolysis of ATP coupled with the translocation of calcium from the cytosol to the sarcoplasmic reticulum lumen
Top3a	7.1E-11	1.13	Shows ATP-independent breakage of single-stranded DNA as a catalytic function
Mitf	2.83E-09	1.5	Regulates the expression of genes with essential roles in cell differentiation, proliferation, and survival. Ruan et al. indicated that Mitf inhibits adipogenesis of primary preadipocytes in ovine
Col1a1	1.03E-083	2.27	The thermogenesis of adipocytes the extracellular matrix and it is shown by Milet et al. that adipose tissue remodelling is associated with several genes including Col1a1 [58]
Cfh	4E-05	3.05	Plays an essential role in maintaining a well-balanced immune response
Bdnf	0.007	3.15	Promotes the differentiation and survival of neuronal populations. Wang et al. identified a leptin-BDNF pathway regulated sympathetic innervation of adipose tissue [59]

Taken together, our analyses indicated a possible alternative splicing event in Usf2, which may cause an alteration in the activity of the regulator and influence pathways such as thyroid and insulin signaling, diabetic cardiomyopathy, and cAMP signaling pathways. In addition to that, our candidate protein, Usf2 is connected to Col1a1, collagen type 1 α, and it is compatible with our enrichment analysis results on intron usage.

Previously, a possible role of Usf2 as a tumor suppressor was reported, proposing that it may be involved in mitochondrial function and energy homeostasis thereby linking Usf2 to conditions such as insulin resistance, type-2 diabetes mellitus, and metabolic syndrome [52]. Another study indicated that Usf2 represses the induction of Carnitine Palmitoyltransferase I β, which is the first step of the carnitine palmitoyltransferase system

and generation of ATP from fat in mitochondria [53] and another study [54] indicated that CLOCK, one of the main regulators of circadian rhythm which is an enriched term in regulators with possible post-transcriptional or translational regulators, regulates Abca1 in macrophages expression using an indirect mechanism involving the transcription factor Usf2. Abca1 protein mediates the secretion of free cholesterol into apolipoprotein A-1 to form high-density lipoprotein, thereby playing a critical role in cholesterol homeostasis. However, none of these studies analyzed Usf2 in the context of alternative splicing and its possible effects on thermogenesis. Our results indicated a possible alternative splicing event on Usf2 that does not affect the expression level of the gene but the activity/function. Hence, we conclude that Usf2 may regulate the browning process in beige adipocytes via the activity of its target genes, and drugs targeting its splicing variants may have therapeutic value in the treatment of obesity such as a drug chemical that increases the activity of Usf2 or targeting the splicing factor that prevents or enhances the alternative splicing events, potentially.

The results can be validated using proteomics and gene knock-out-based transcriptomics approaches for further investigation. Integration of these kinds of multi-omics studies computationally can increase the resolution and our understanding of the browning mechanism. Also, single cell RNA-Seq (scRNA-Seq) studies have the potential to identify the alterations in a perspective of trajectory and identify heterogeneous cell populations.

In summary, machine learning-based detection of splicing events combined with detection of aberrant regulatory events with RREA of cold exposure and browning in adipose tissue has shed new light on the intricate regulatory mechanisms that control metabolic processes. We reported alternative splicing events and their downstream regulatory effects in browning processes the first time in the literature as an alternative to methods already reported [60]. With the accumulation of high-quality data and the development of more sophisticated machine/deep learning tools for the identification and classification of differential events in datasets, such analyses are likely to provide increasingly useful information for advancing our understanding of the fundamental and targetable processes that regulate metabolism in health and disease.

5. Acknowledgements

The authors declare no conflict of interest. The authors declare that all results are obtained computationally. Results from any experimental study were not reported.

6. Code availability

R scripts used throughout the study are available at: https://github.com/hkarakurt8742/adipose_browning

7. Supplementary Materials

The supplementary figures and tables can be found at: https://aperta.ulakbim.gov.tr/record/263303

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