Pangenomic cDNA Microarray Profiling of Human Skeletal Muscle Gene Expression during Epinephrine Infusion

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ABSTRACT

Epinephrine, the stress hormone secreted by adrenals, has a profound impact on energy expenditure, protein and carbohydrate metabolism in skeletal muscle. β_2-adrenergic receptor-mediated activation of cAMP-dependent signal transduction modulates gene transcription by the cAMP response element binding protein family of transcription factors. Very little is known however on the target genes of epinephrine in human skeletal muscle. Here, we determined mRNA expression profiles before and during a 6-h epinephrine infusion performed in 9 young men. The increased plasma level of epinephrine elicited an increase in resting metabolic rate, glycemia and free fatty acid level without change in norepinephrine level. Stringent statistical analysis of data obtained using 43,000 cDNA element microarrays showed that 1206 and 474 genes were up and downregulated, respectively. Microarray data were validated using reverse transcription-quantitative PCR. Gene classification was performed through data mining of Gene Ontology annotations, cluster analysis of regulated genes among 14 human tissues and correlated variations of mRNA and clinical parameter levels. Key genes of the cAMP-dependent transcription pathway and genes with known functional cAMP response elements were regulated by the hormone. The impact on metabolism was illustrated by coordinated regulations of genes involved in glycogen turnover and glycolysis and in protein breakdown. Epinephrine had a profound effect on genes involved in immunity and inflammatory response. Information on 526 mRNAs corresponded to expressed sequenced tags or represented genes encoding hypothetical proteins of unknown function. These data define the molecular signatures of epinephrine action in human skeletal muscle.

INTRODUCTION

Catecholamines are involved in a host of physiological functions in particularly those integrating responses to stress signals. Historically, catecholamines have been associated with the “fight-or-flight” response. Norepinephrine is the major neurotransmitter in the peripheral sympathetic nervous system whereas epinephrine is the primary hormone secreted by the adrenal medulla in humans. As part of the response to stress, release of both the neurotransmitter and the hormone may be stimulated. Their effect on target tissues is mediated by 6 α and 3 β-adrenoceptor subtypes (1). The variety of receptors and signal transduction pathways combined with differential tissue distribution accounts for the diversity of biological responses. Epinephrine acts both on α and β-adrenoceptors. Intravenously, it evokes an increase in blood pressure that is explained by a direct myocardial stimulation through β-adrenoceptors and a vasoconstriction in many vascular beds through α-adrenoceptors. However, blood flow is markedly increased in skeletal muscle through the powerful β_2-adrenoceptor-mediated vasodilation. The hormone also influences a number of important metabolic processes (2). It decreases the uptake of glucose in peripheral tissues, partly through an inhibition of insulin secretion. It stimulates glycogenolysis in several organs and has a well characterized effect on adipose tissue lipolysis that allows modulation of plasma free fatty acid levels (3). Epinephrine stimulates energy expenditure in humans. This effect is mediated by β_1- and β_2-adrenoceptors (4). The sympathetically mediated thermogenesis may be explained by the increase in myocardial energy expenditure, increase in adipose tissue lipolysis and increase in substrate oxidation, most notably in skeletal muscle. Catecholamines have also a profound effect on protein metabolism in skeletal

muscle (5). Treatment with β_2-adrenergic agonists induces hypertrophy of skeletal muscle in livestock and humans. Changes in skeletal muscle mass may be due to an increased rate of protein synthesis and a reduced rate of protein breakdown.

Skeletal muscle is equipped uniquely with the β_2-adrenoceptor that accounts for the effect of epinephrine on the tissue. Through activation of adenylyl cyclase, stimulation of the Gs-protein-coupled receptor leads to an increase of intracellular cAMP and activation of protein kinase A (PKA). This signaling cascade, one of the most versatile and multifunctional, is responsible for the modulation of numerous processes including gene transcription. Phosphorylation of the cAMP response element binding protein (CREB) stimulates cellular gene transcription of target genes (6,7). The stoichiometry of CREB phosphorylation correlates well with intensity of stimulus and the level of gene activation. The CREB family transcription factors, i.e. CREB, cAMP response element modulator (CREM) and activating transcription factor 1, belong to the basic domain-leucine zipper class of transcription factors. CREB dimers bind to cAMP response element (CRE) often represented by the consensus palindrome TGACGTCA. The CRE is usually located in the proximal promoter region. Binding of CREB promotes recruitment of RNA polymerase II complexes. Instrumental in the activation of transcription is the bipartite CREB transactivation domain which is composed of the constitutive Q2 domain and the kinase-inducible domain. Transcriptional repression involves dynamic dephosphorylation of the activators or involvement of the CREM isoform ICER (inducible cAMP early repressor). ICER expression is strongly induced by cAMP and ICER itself is able to repress its own expression. These dynamic and versatile processes allows fine tuning in the control of gene expression by catecholamines.

Considerable knowledge has been gained on the molecular mechanisms of cAMP dependent modulation of gene transcription. However, a limited number of target genes have been identified. As skeletal muscle is an important site of action for epinephrine, we wished to analyze global transcriptional modifications induced by short-term exposure to the hormone.To that hand, we used a microarray with 43,000 cDNAs that represent a large fraction of the human transcriptome and measured the changes before and after a 6 h infusion with epinephrine. We report here that epinephrine directly modulates the mRNA levels of 1680 genes in human skeletal muscle. Most of the genes are novel targets of epinephrine. They belong to functional classes that explain the biological and metabolic effect of epinephrine.

RESULTS AND DISCUSSION

Effect of epinephrine on cardiovascular and metabolic parameters

Nine healthy male volunteers received a 6h infusion of epinephrine at 0.04 $\mu g.kg^{-1}.min^{-1}$. The clinical parameters are shown in Table 1. The infusion induced a 30-fold increase in plasma epinephrine levels without modifications of plasma norepinephrine levels. The levels obtained with epinephrine correspond to physical exercise at maximal intensity (8). The increase in heart rate was significant at 3 and 5 h. Systolic and diastolic blood pressures were not modified by the infusion. The resting metabolic rate adjusted for lean body mass was significantly increased by about 20%. Skeletal muscle has been shown to account for 40% of epinephrine-induced thermogenesis (9). As an index of the stimulation of adipose tissue lipolysis by catecholamines (3), plasma glycerol and non-esterified fatty acid levels were

increased during epinephrine infusion. The effect on non-esterified fatty acid concentrations was characterized by a peak at 1 h followed by a sustained elevation of plasma levels compared to basal values albeit at lower levels than at 1 h. The peak may be related to the surge in fatty acids released by adipose tissue. At subsequent time points, fatty acid concentrations may reflect the continuously stimulated lipolysis balanced by an increase in fatty acid utilization. This increase is not the result of a change in the ratio between lipid and carbohydrate oxidation as the respiratory quotient was not modified. However as energy expenditure was increased, lipid oxidation was induced. The increase in blood levels of glucose reflects the stimulation of hepatic glycogenolysis (2). The elevated glycemia may induce the accompanying increase in plasma insulin levels. When compared to the effect of fasting, the increase in plasma insulin levels is lower than expected for the observed variation in glycemia. This less than expected induction is probably due to the well known direct inhibitory action of catecholamines on insulin secretion (1).

Changes in mRNA expression induced by epinephrine

Amplified RNA (aRNA) from skeletal muscle were used to hybridize high density microarrays with 42878 cDNAs. Accurate measurements of net spot intensities were recovered for 37642 cDNAs. High intensity signals for at least 8 over 9 experiments were obtained for 16739 cDNAs (43%) which were selected for further analysis. Using conventional t-test with a $p=0.01$, one would expect to identify 170 genes by chance. To control for multiple testing, we used Significant Analysis of Microarray (SAM), a permutation-based method that determines a false discovery rate (FDR), i.e. the probability that a given gene identified as differentially expressed is a false positive (10). The procedure resulted in the selection of 2082 cDNAs as being

differentially expressed with a FDR of 0.1%. The very low FDR corresponds to a median number of falsely called genes of 2 with a 90^{th} percentile of 70 genes. This FDR is much lower than the FDR of 15% which we reported in our study on thyroid hormone (11). The improved confidence in the present study probably comes from the increased number of subjects analyzed in microarray experiments (9 vs. 5) but also from a normalization procedure that uses locally weighted linear regression (lowess). Lowess is a method that can remove the dependence on intensity of the $\log_2$(ratio) values (12). Our study design also contributes to the obtention of statistically significant results. Longitudinal studies of hormone effect on skeletal muscle gene expression have yielded lists of significant genes with control for multiple testing despite changes in gene expression that are relatively modest (11,13) and present study). Transversal studies comparing gene expression profiles in skeletal muscle of diabetic and non diabetic subjects failed to identify significant genes when multiple comparisons are taken into account (14,15). The variability between individuals is probably less critical in longitudinal studies as each individual is its own control. Among the 2082 cDNAs, 1107 were represented by a unique clone on the microarray. 870 cDNAs had replicates among the 37642 cDNAs. We tested whether the expressed values of the $\log_2$ ratio of replicates was 0. 402 genes were eliminated using the Student's *t*-test ($p<0.05$). The final list (Supplementary Table 1) comprised 1680 differentially expressed genes, 1206 being upregulated (mean fold change 1.37) and 474 downregulated (mean fold change 0.71). It must be stressed that this selection procedure is highly stringent because each mRNA variants of a gene produced by alternative promoters, splicing or polyadenylation sites have the same UniGene number but are not necessarily identically regulated.

Validation of microarray data

We first checked whether, in our experimental conditions, mRNA amplification did not induce a distortion in mRNA representativity. mRNA levels of 3 genes was quantified using reverse transcription-real time PCR (RT-qPCR) on both total and aRNA from 6 patients. The correlation was very high (r=0.99) indicating, as previously reported (16,17), that aRNA are representative of mRNA in total RNA preparations. In order to confirm data obtained from microarray hybridizations and statistical analysis, we measured changes in mRNA levels for 14 genes, including 3 up, 4 downregulated and 7 unaffected genes sorted by SAM. These genes are representative of various biological processes. Table 2 shows the comparison between the fold changes in mRNA levels measured with microarray hybridization using aRNA and the fold changes calculated using RT-qPCR starting from total RNA. The estimation of the gene expression ratios by the microarray experiments highly correlated with the data obtained by RT-qPCR (r=0.97). Statistical results were discordant for 2 genes, secreted protein acidic and rich in cysteine (SPARC) and voltage-dependent anion channel 1 (VDAC1). The SPARC gene was selected by SAM in microarray experiments and exhibited borderline significance ($p<0.1$) in RT-qPCR experiments. On the contrary, VDAC1 mRNA levels which were different using RT-qPCR were not found differential with the microarray data. This latter result shows that the stringent statistical procedure aimed at decreasing the number of false positives discards many genes that are differentially expressed.

Global view at mRNA expression changes induced by epinephrine

The majority of the genes (70%) was upregulated. Most of them may use a classical transcriptional activation process mediated by an increase in intracellular cAMP level and activation of members of the CREB family (CREB, CREM and activating transcription factor 1). However, the transcriptional activity of AP-2, NFκB and some nuclear receptors is modulated by cAMP (6). These factors may thereby contribute to the positive gene regulation. Nearly 30% of the genes were downregulated. The mechanisms underlying cAMP-mediated transcriptional repression remain obscure. One pathway could involve muscle-specific members of the basic helix-loop-helix family such as MyoD and Myf5 which are inhibited by PKA (18). Phosphorylation and activation of transcriptional (co)repressors is another possibility. In yeast, PKA-mediated phosphorylation of the CREB repressor Sko1p induces the nuclear translocation and thereby the activity of the factor (19). Phosphorylation of histone deacetylases by PKA has been reported (20) but it is unclear whether it affects transcription repression. Ranking of variations in mRNA and epinephrine levels according to Spearman correlation coefficients allowed a selection of the regulated genes most closely associated with changes in epinephrine concentrations. A graphical representation (Fig. 1) is shown for the top 15 genes up ($0.6<r<0.8$) and downregulated ($-0.8<r<-0.5$). The tight association between the parameters suggests that these genes which 18 have yet unknown functions are direct targets of epinephrine and thereby constitute good candidates for a deeper analysis of transcriptional mechanisms of regulation.

To sort the regulated genes into functional categories, we used Gene Ontology (GO) annotations (www.geneontology.org) and an in-house recently developed data mining tool. Figure 2 shows the relative impact of the hormone on genes defining representative functions and its effect on gene induction or

repression. GO annotations were available for 12655 genes represented on the microarray and 999 regulated genes. The main functional categories at level 3 of the biological process tree were metabolism, cell growth and maintenance and, cell communication, i.e. cell-cell and cell-extracellular matrix interactions. Upregulated genes were overrepresented in the response to external stimulus, response to stress and cell motility categories (87, 76 and 71 %, respectively). For 526 cDNAs, available information indicated expressed sequence tags or genes encoding hypothetical proteins of unknown function. The genes represent novel putative target genes of catecholamines. Comparison of mRNA expression patterns for epinephrine-regulated genes in 14 tissues (21) showed a cluster containing 65 genes highly expressed in the cardiac muscle (Fig. 3A). Among them, 21 genes have also been shown to be expressed in skeletal muscle (source.stanford.edu and telethon.bio.unipd.it/GETProfiles/) (22). The cluster is likely to represent a set of genes typical of striated muscles. Catecholamines and thyroid hormones both increase resting metabolic rate, an effect that is mediated in part by action on skeletal muscle (9,23). We therefore compared epinephrine-regulated genes with the 381 upregulated and 2 down regulated genes that we have recently shown as being controlled by triiodothyronine in human skeletal muscle (Supplementary Table 2) (11). Twenty-one genes had discordant (up under triiodothyronine and down under epinephrine) and 12 concordant regulations. The p85α regulatory subunit of phosphatidylinositol 3-kinase, a key enzyme of insulin signaling, is upregulated to the same extent by both types of hormones in line with their antagonistic effect of insulin action. However, the comparison shows that the overlap in terms of hormonal gene regulation is limited. It is therefore likely that the transcriptional adaptations

leading to similar physiological effects (e.g., on energy expenditure, substrate oxidation and protein synthesis) impact distinct genetic networks.

cAMP-dependent signal transduction pathway

Epinephrine is acting on skeletal muscle through the Gs-coupled β_2-adrenergic receptor and stimulates cAMP production. We examined the effect of the hormone on expression of the genes involved in the cAMP signaling pathway (Fig. 4). At the level of adenylyl cyclase, the enzyme that converts ATP into cAMP, there was a down regulation of adenylyl cyclase-associated protein 2, a protein that links the adenylyl cyclase protein complex to the actin cytoskeleton. Several phosphodiesterases (PDE) which degrade intracellular cAMP and hence play a crucial role in the modulation of cAMP signaling were regulated by epinephrine. The cAMP-specific PDE4B, the cGMP-stimulated PDE2A and the cGMP-specific PDE6A and PDE6G were all upregulated (24). PDE4 and PDE2 contribute to cAMP hydrolysis whereas PDE6 which is expressed in retinal rod but also in skeletal muscle hydrolyzes cGMP. The PDE4D interacting protein also called myomegalin was downregulated. Expressed in skeletal muscle, myomegalin functions as an anchor to localize PDE4D to the Golgi/centrosomal region (25). This coordinated upregulation may constitute a feedback mechanism to control cAMP levels but also reveals the crosstalk between cAMP and cGMP pathways. PKA exerts a pivotal role in the cAMP signaling pathway through the phosphorylation of proteins involved in numerous cellular processes. Several families of proteins interact with PKA and modify its location, and hence biological role, within the cell. The β inhibitor of PKA which inactivates PKA through binding to the catalytic subunit in the nucleus and

transfer to the cytoplasm was downregulated. Conversely, expression of the PKA anchor protein 4 which binds to the regulatory subunit was induced. Seven subunits of protein serine/threonine phosphatases were regulated during epinephrine infusion. On the five genes encoding catalytic or regulatory subunits of protein phosphatase 1 and 2A that may play an important role in the control of CREB (de)phosphorylation (6), all but one, a regulatory subunit, showed decreased mRNA expression.

Modulation of transcription by the cAMP signaling pathway is mainly ensured by the CREB family of transcription factors. Consistent with the presence of CRE in its promoter, CREB expression was upregulated by epinephrine. A crucial phase in the transcriptional activation is the recruitment by CREB of the coactivator CREB binding protein (26). CREB binding protein inhibitory protein 1 was downregulated by epinephrine. This inhibitor is highly expressed in skeletal muscle where it inhibits MyoD-dependent transcription (27). The decreased expression of the coactivator inhibitor may favor both CREB action and MyoD-dependent transcription of skeletal muscle-specfic genes. We also observed an induction of CREM/ICER mRNA. CREM/ICER is an early response gene which burst of expression is transient due to a negative autoregulatory loop (28). By analogy with other organs, CREM/ICER induction in skeletal muscle may cause a refractory period for cAMP inducibility of some genes.

The overall picture that may be deduced from the changes in the cAMP-dependent signaling cascade is a combination of attenuation and stimulation. Attenuation of cAMP effect is illustrated by PDE and CREM/ICER upregulation. Reinforcement of cAMP action may be contributed through positive effect on CREB expression and downregulation of serine/threonine phosphatases.

cAMP-regulated genes containing cAMP response elements

CREB mediates the activation of cAMP responsive genes by binding as a dimer to the consensus palindrome TGACGTCA. Variant functional CRE containing the half-site TGACG have been identified but may be less active. Using recently published surveys, we wished to determine whether genes regulated by epinephrine in skeletal muscle had been previously identified as genes with functional CRE. Mayr and Montminy have produced a list of cAMP-regulated genes containing consensus sites for CREB binding (26). Using a hidden Markov model trained on known CREB sites, Conkright et al. performed a genome-wide analysis of target genes (29). Conservation between human and mouse sequences was used to diminish spurious occurrence of CRE. The list covered genes with a wide range of functions and tissue specificity. However, many of the identified genes were expressed in neurons. Comparison of the two lists of genes with our data identified 12 genes (Table 3). All were upregulated during epinephrine infusion. Besides CREB and CREM/ICER, the table contains the dual specificity phosphatase 1 which is a known CREB target gene also identified in the genome wide analysis and 3 transcription factors (aryl hydrocarbon receptor, Pit 1 and PBX2). This comparison illustrates the potential of bridging information from in vivo hormonal effect on gene expression and molecular mechanisms of gene transcription.

Energy metabolism

Carbohydrates are stored as glycogen which catabolism provides a direct source of energy for the exercising muscle. Thirty genes involved in carbohydrate metabolism are regulated (24 up and 6 down) (Table 4). Several target genes were involved in glycogen metabolism including 5 of the 6 downregulated genes (Fig. 5). In liver and skeletal muscle, UDP-glucose is a direct precursor of glycogen. UDP-glucose pyrophosphorylase 2 transfers a glucose moiety from glucose-1-phosphate to MgUTP and forms UDP-glucose. The energy of the phospho-glycosyl bond of UDP-glucose is utilized by glycogen synthase to catalyze the incorporation of glucose into glycogen. The glucan (1,4-alpha-) branching enzyme 1, a monomeric enzyme highly expressed in liver and muscle, creates alpha 1,6- glucosidic linkages between a terminal fragment of 6-7 glucose residues to an internal glucose residue at the C-6 hydroxyl position allowing ramification of the glycogen macromolecule. The glycogen synthase kinase 3 beta was upregulated. This enzyme is a cAMP-dependent serine/threonine kinase inactivating glycogen synthase (30). Regarding glycogen breakdown, the brain isoform of the glygogen phosphorylase was induced under epinephrine infusion while the mRNA for the cAMP-activated phosphorylase β kinase was decreased. Defects in phosphorylase β kinase are the cause of phosphorylase kinase deficiency of liver and muscle, a glycogen storage disorder (31). A catalytic and a regulatory subunits of protein phosphatase 1 were also negatively regulated. Protein phosphatase 1-mediated dephosphorylation relays insulin action in the inhibition of glycogenolysis and activation of glycogen synthesis (32). These regulations concur to an increase in glycogenolysis and a decrease in glycogen synthesis.

Fructose-2,6-bisphosphate has a central role in glucose metabolism. It plays antagonistic actions between glycolytic and gluconeogenic pathways (33). The 6-

phosphofructo-2-kinase/fructose-2,6-biphosphatase is one of the most highly upregulated gene of the present study. The corresponding protein is a homodimeric bifunctional enzyme involved in the close regulation of the levels of fructose 2,6-bisphosphate which is a potent allosteric activator of 6-phosphofructo-1-kinase and reduces the activity of the key regulatory enzyme of gluconeogenesis, fructose-1,6-bisphosphatase. The steady state concentration of fructose-2,6-bisphosphate is determined by the balance between these opposing reactions. Adenovirus-induced overexpression of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase in mouse liver has been shown to result in reduced activity of glucose-6-phosphatase and stimulation of glucokinase, thereby stimulating the glycolytic rate (34). Furthermore, two genes encoding proteins involved in galactose metabolism were upregulated, the UDP-galactose transporter UGT1 and the UDP-glucose ceramide glucosyltransferase-like 2. Coming from lactose, galactose is converted to UDP-galactose prior to glucose metabolism. Altogether, these regulations are in favor of an increase in anaerobic glycolytic metabolism indicating a rapid adaptive effect of catecholamines on human skeletal muscle metabolism to support high transient energy demand.

Upregulated genes involved in lipid metabolism comprised several apolipoproteins. The apolipoprotein C-II, a component of the very low density lipoprotein fraction in plasma, is a necessary cofactor for the activation of lipoprotein lipase, the gate keeper of fatty acid supply to skeletal muscle. Apolipoproteins L1 and M are associated with high density lipoproteins. Apo C-I functions as a recognition signal for the cellular binding and internalization of low density lipoprotein particles by the apo B/E receptor. In addition, the low density lipoprotein receptor gene was upregulated. This receptor binds low density lipoproteins, the major cholesterol-

carrying lipoproteins of plasma, and transports it into cells by endocytosis. Another critical point in lipid metabolism is the fatty acid entry into the cell. We found that two genes encoding plasma membrane fatty acid transport proteins are regulated. The fatty acid transporter 4 which is highly expressed in skeletal muscle is a member of the transmembrane protein family that enhances long chain fatty acid uptake. However, the fatty acid transporter FAT/CD36 was downregulated. These opposite regulations may sign a change in the characteristics of fatty acid entry into skeletal muscle (35).

Few genes participating in the mitochondrial respiratory chain were regulated by epinephrine. Two mRNAs encoding proteins involved in the transfer of electrons from NADH to the respiratory chain were upregulated, a subunit of the NADH dehydrogenase (ubiquinone) 1 and a subunit of the NADH dehydrogenase (ubiquinone) flavoprotein 1. A component of the ubiquinol-cytochrome c reductase complex (complex III), the Rieske iron-sulfur polypeptide 1 and the β polypeptide of the H^+ transporting mitochondrial F1 complex ATP synthase were downregulated. The role of skeletal muscle uncoupling proteins is still elusive (36). They may play a role in energy expenditure through uncoupling of ATP synthesis from O_2 consumption, mitochondrial fatty acid metabolism and control of reactive oxygen species production. Expression of uncoupling proteins 2 and 3 was not altered by epinephrine as confirmed by quantitative RT-qPCR measurements (Table 2). However, an induction of uncoupling protein 4 which is upregulated during cold exposure in brain (37) was observed. PGC-1α is a transcriptional coactivator that plays an important role in the integration of responses to thermogenic stimuli (38). It is induced in rodent skeletal muscle following sympathetic nervous system activation. We found no change in mRNA expression which may indicate a difference in PGC-

1α gene regulation between rodent and human skeletal muscle (Table 2). Compared to thyroid hormone (11,39), the transcriptional effet of catecholamine on the respiratory chain is moderate. It seems therefore likely that catecholamine effects on skeletal muscle metabolic rate use different pathways. An indication of potential genes involved in energy metabolism may be provided by the comparison between variations in gene expression and changes in resting metabolic rate (Fig. 6). Ranking of genes classified in metabolism from GO annotations using Spearman correlation coefficients revealed 20 genes upregulated with $r>0.8$. The list includes 9 transcription factors that may play a role in the regulation of energy expenditure. Alternatively, the 20 genes may constitute potential markers of changes in energy expenditure as their mRNA variations parallel changes in resting metabolic rate.

Protein degradation

Catecholamines have an anabolic effect on skeletal muscle protein metabolism (5). Epinephrine inhibits proteolysis in human skeletal muscle (40). Intracellular proteolysis occurs via a lysosomal Ca^{2+}-dependent and a non-lysosomal ATP-dependent pathway. The latter system degrades most endogenous proteins through covalent linking of proteins to the ubiquitin system then rapid degradation by the proteasome machinery. It is striking that most of the downregulated genes involved in protein breakdown participated in the ubiquitin system. Three genes encode members of the F-box protein family. The F-box proteins constitute one of the four subunits of the SKP1-cullin-F-box ubiquitin protein ligase complex. This protein plays a unique role in the phosphorylation-dependent ubiquitination. In addition, the ring-box 1 mRNA expression was reduced. The gene encodes a protein that

heterodimerizes with cullin-1 to catalyze ubiquitin polymerization. The mRNA level of ubiquitin protein ligase E3A was decreased by epinephrine. Ubiquitin ligases play a crucial role in skeletal muscle atrophy and β-adrenergic agonists are well-known to reduce muscle wasting (41). Such regulations underline the molecular basis for an anticatabolic impact of catecholamines.

Regulation of genes involved in defense response

It is well established that inflammation activates the sympathetic nervous system. However, it is also increasingly recognized that catecholamines modulate the immune system and inflammatory response (42). Figure 2 revealed that that most genes involved in the response to stress (level 3 of biological process GO annotations) were upregulated. To refine the classification, epinephrine-regulated genes were selected for the defense response (level 5 of biological process GO annotations). All but one were induced (Supplementary Table 3). On 96 genes, 35 encode secreted proteins. A coordinated regulation of genes encoding chemokines with Cys-Cys motif is apparent as 10 members (CCL1, 4, 5, 7, 11, 13, 14, 18, 19 and 20) were positively regulated on a total of 17 genes of the family represented on the array. Cys-X-Cys chemokines (CXCL4 and 10) were also upregulated. These secreted cytokines are involved in immunoregulatory and inflammatory processes. They are chemotactic factors that possess various specificities to attract monocytes, lymphocytes, basophils, eosinophils and neutrophils. Other secreted peptides with comparable functions were also upregulated such as interleukin 16, defensin 4, the tumor necrosis factor ligand superfamily member 7 and the endothelial monocyte-activating polypeptide. Defense against pathogens is also ensured by the

complement system. Epinephrine induced mRNA expression of components of the classical (1, 2, 5 and 8β) and alternative (B and D) pathways as well as associated or related peptides (C4 binding protein β and vitronectin). Pleiotropic effect on the immune system was also illustrated by the upregulation of interleukin receptors (IL1R accessory protein, IL2Rγ, IL4Rα and IL6Rα). To get another view at the effect of epinephrine on cell defense mechanisms, we analyzed the pattern of epinephrine-regulated mRNA expression in leukocytes and tissues producing lymphocytes (Fig. 3B). The cluster comprised 69 genes highly expressed in these tissues. Analyses of gene function and tissue distribution bring independent evidence that epinephrine has a profound impact on inflammatory response in skeletal muscle. This strong response may have paracrine effects through secretion of cytokines and contribute to the increase of energy expenditure through the installation of an inflammatory state.

Conclusion

In this paper, we have characterized the skeletal muscle transcriptional profile of the response to epinephrine in vivo. Sorting of the regulated genes into functional classes and determination of common expression patterns in human tissues defined the molecular signatures that underlie the effects of epinephrine in human skeletal muscle. Epinephrine modulated the expression of genes involved in the cAMP-mediated transcription pathway, thereby promoting autoregulatory mechanisms. In line with the known physiological effects of the hormone, modulation of genes involved in glycogen and glucose metabolism and protein catabolism was observed.

The study also revealed novel targets, especially genes involved in the immune system and inflammatory response.

MATERIAL AND METHODS

Subjects

Nine healthy male Caucasian volunteers (22–32 years old) who had not been submitted to any pharmacological or nutritional protocols before the study were recruited. All had stable weight during the previous 3 months. Their body mass index was 23.5 ± 0.5 kg/m2 (range 20.8-25.7 kg/m^2). Selection of subjects was based on a screening evaluation consisting of detailed medical history, physical examination, complete blood count, urine analysis, resting electrocardiogram, blood pressure measurements, and several blood chemistry analyses. The subjects were on their usual diet before the study, and none were engaged in heavy physical activity training. The study protocol was approved by the Comité Consultatif de Protection des Personnes Toulouse 1 (Ethics Committee), and written informed consents for all subjects were obtained. Investigations were performed at the Clinical Investigation Center of Toulouse University Hospitals.

Experimental protocol

An initial screening visit was performed seven days before the beginning of the experimental protocol to check the inclusion criteria. Body composition was assessed in fasting condition by dual-energy X-ray absorptiometry performed with a

total body scanner (DPX, software 3.6 Lunar Radiation Corp., Madison, Wisc.) enabling quantification of fat mass, lean body mass and total bone mineral content, the day before the experimental day (43). The same investigations were performed before and after epinephrine infusion. After an overnight fast, a catheter was inserted at 8 a.m. into the left antecubital vein for blood sampling and kept patent with isotonic saline. After a one-hour resting period in supine position, oxygen consumption (VO_2) and carbone dioxide production (VCO_2) were monitored over 30 min by using an open-circuit ventilated-canopy system (Deltatrac II monitor, Datex Instrumentarium Corp., Helsinki, Finland) calibrated with a reference gas. Resting metabolic rate was derived from VO_2 and VCO_2 by using indirect calorimetry (44). After resting metabolic rate measurement, three 10-min-interval blood samples were drawn for determinations of basal hormonal and metabolic parameters. Then a percutaneous biopsy of the vastus lateralis muscle was obtained by using Weil Blakesley pliers (45). Approximately 3 ml 1% lidocaine was injected into the skin before the biopsy. The procedure involved a 5-mm incision through the skin and muscle sheath 15–20 cm above the knee. Average 50-mg (wet weight) muscle samples were obtained, immediately frozen in liquid nitrogen, and stored at -80°C until analysis. The epinephrine infusion was performed through the intravenous catheter placed in the right arm by using an Auto-Syringue infusion pump. Epinephine with isotonic saline as vehicle was infused intravenously at 0.04 $\mu g.kg^{-1}.min^{-1}$ during 6 hours. One h-interval blood samples were drawn from the catheter placed in the left arm for determinations of hormonal and metabolic parameters during the infusion. During the baseline period and epinephrine infusion, the heart rate was continuously recorded by using a standard three-lead electrocardiogram, and systolic and diastolic blood pressures were evaluated every 10 min by using a

Dinamap device. After epinephrine infusion, skeletal muscle biopsies were performed on the controlateral side.

Biochemical determinations

Plasma catecholamines were assayed by high-pressure liquid chromatography by using electrochemical (amperometric) detection, as previously described (46). The detection limit was 20 pg/sample for both catecholamines. Day-to-day and within-run variabilities were 4% and 3%, respectively. Glycerol was determined in plasma by using an ultrasensitive radiometric method (47); the intra-assay and interassay variabilities were 5.0 and 9.2%, respectively. Plasma glucose was assayed with a glucose oxidase technique (Biotrol, Paris, France); the intra-assay and interassay variabilities were 1.5 and 5.1%, respectively. Nonesterified fatty acids were assayed with an enzymatic method (Unipath, Dardilly, France); the intra-assay and interassay variabilities were 1.1 and 1.6%, respectively. Plasma insulin was measured by using a Bi-insulin IRMA kit from Sanofi Diagnostics Pasteur (Marne-La-Coquette, France); the intra-assay and interassay variabilities were 2.7 and 5.8%, respectively. Phenotypical values are given as mean $\pm$ SEM. One way analysis of variance for repeated measures was used for comparisons of the metabolic and hormonal parameters before and during the epinephrine infusion with time as the factor of analysis, followed by a Bonferroni-Dunnett post hoc test with baseline values as the control. $p < 0.05$ was the threshold of significance.

Determination of mRNA levels

Total RNA was extracted using the RNA STAT-60 isolation reagent (Tel-Test). Total RNA quantity and quality were assessed using the Agilent 2100 bioanalyzer and RNA 6000 labChip Kit (Agilent Technologies, Massy, France). Reverse transcription (RT) was performed using Thermoscript reverse transcriptase (Invitrogen) and random hexamers with 500 ng of total RNA for each sample from the nine patients. cDNA (10 ng) was used as template for real-time PCR. Real time PCR was performed on GeneAmp 7000 Sequence Detection System (Applied Biosystems) as previously described (11,48). A standard curve for each primer pair was obtained using serial dilutions of human skeletal muscle cDNA. We used 18S ribosomal RNA as control to normalize gene expression using the Ribosomal RNA Control TaqMan Assay kit (Applied Biosystems).

RNA amplification

Antisense RNA (aRNA) were prepared using the Message Amp aRNA Kit (Ambion, Cambridgeshire, UK). This amplification procedure which is not based on PCR has previously been validated (16,17). It does not distort the relative abundance of individual mRNAs. Briefly, 400 ng of total RNA were used for an RT reaction containing a T7 promoter sequence primer. After second strand cDNA synthesis, *in vitro* transcription was performed overnight resulting in a approximately 1000 fold yield in aRNA. aRNA quantity and quality were assessed using the Agilent 2100 bioanalyzer.

Probe labeling and microarray hybridization

Fluorescent probes were synthesized from 3 µg of aRNA using the CyScribe First Strand cDNA Labelling Kit (Amersham Biosciences, Orsay, France). Cy3 and Cy5 probes from one subject were purified and concentrated using Microcon YM-30 column (Millipore) after the addition of human cot-1 DNA (Invitrogen), yeast t RNA and poly(dA)DNA (Sigma). After denaturation, the probe was added to the array in a 3.4 X SSC 0.3% SDS buffer which was covered by a glass coverslip. The slide was then placed in a sealed humidified hybridization chamber for a 16 h hybridization at 65°C. Slides were washed twice in 2 X SSC 0.1% SDS, 1 X SSC then, 0.5 X SSC. The arrays were immediately scanned using a GenePix 4000A scanner (Axon Instruments). Images were analyzed using GenePix pro 3 software. Data files generated by Genepix were entered into the Stanford Microarray Database (genome-www5.stanford.edu/MicroArray/SMD/). After a filtering procedure omitting manually flagged elements (i.e. bad quality spots) and spots with an average intensity below 2.5-fold above the background, 39,141 spots were recovered. A uniform scale factor was applied to all measured intensities in order to normalize signal intensities between both images. The $\log_2$ Cy5/Cy3 ratios (epinephrine/control) were extracted for the 9 experiments. Prior to calculations, the data from at least 8 over 9 experiments were normalized using lowess (12). Data were analyzed using the SAM procedure (www-stat.stanford.edu/~tibs/SAM/), a validated statistical non parametric technique for identifying differentially expressed genes across high-density microarrays (10,11). A one class analysis was performed and additional criteria were imposed to increase confidence that the estimated changes observed for one cDNA reflect real differences in gene expression. The additional analysis consisted in the identification of other clones representing the same gene (that is the same UniGene number) as the one sorted using SAM. For

each gene, Student's *t*-test was performed for each replicates. To analyze the function of the known genes, we used GO annotations (49) (www.geneontology.org) and a newly in-house developped data mining tool. To determine whether epinephrine-regulated genes shared common patterns of expression, cluster analysis of the transcripts was performed among 14 different human tissues using a hierarchical clustering method (rana.lbl.gov/EisenSoftware.htm) (50).

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Figure legends

Fig. 1. Graphical representation of changes in gene expression and epinephrine levels during hormonal treatment.

Up (left panel) and down (right panel) regulated genes with the 15 highest Spearman correlation coefficients are shown. Each line corresponds to one gene identified by UniGene number. Variation in mRNA levels is shown on the x axis. Each dot represents one subject. The colour of the dot corresponds to the extent of the variation in epinephrine levels (∆epinephrine/epinephrine).

Fig. 2. Impact of epinephrine on genes grouped in functional categories.

Gene Ontology (GO) annotations (www.geneontology.org) were used to depict the main functions modified by epinephrine with an in-house-developed program. GO numbers are indicated for each function. Blue bar shows the number of microarray genes at a GO level (shown in insert) divided by the number of annotated microarray genes. Purple bar shows the number of regulated genes at a GO level (shown in insert) divided by the number of regulated and annotated genes. Black bar shows the number of regulated genes at a GO level (shown in insert) divided by the number of microarray genes at the same GO level (shown in insert). Red bar represents the ratio of the percentage of upregulated genes at a GO level on all upregulated genes divided by percentages for up and downregulated genes. Green bar is a similar representation for down regulated genes. The number of up and downregulated genes with annotations are shown as inserts within red and green bars, respectively.

Fig. 3. Two-dimensional clustering of 14 tissue experiments and transcripts which showed variation during epinephrine infusion.

The set of genes were selected from the data matrix provided by the hybridization of 14 human tissues to a common reference pool. Experiments and responsive genes were grouped by hierarchical clustering after centering the $\log_2$ ratios on the mean for all experiments. Each row represents a single gene and each column an experimental sample. For each sample, the ratio of the abundance of the transcripts of each gene to the mean abundance across all experiments is represented by the color of the corresponding cell in the matrix file. Green boxes represent transcript levels lower than the mean. Red boxes are transcript levels higher than the mean, black boxes transcript level equal to the mean and gray lines are missing data. Each node of the gene dendrogram was analyzed and we focused on sets of genes clustered by functions. The upper dendrogram shows similarities in the expression pattern between tissues. A, Genes highly expressed in heart. Genes bosed in green have been shown to be expressed in skeletal muscle. B, Genes highly expressed in leukocytes and lymphocyte-producing tissues. Genes boxed in green encode secreted peptides.

Fig. 4. Effect of epinephrine on the expression of genes of the cAMP signal transduction cascade.

Filled circles and open ellipses represent genes down and upregulated, respectively. Gs, stimulatory G protein; ACAP, adenylyl cyclase-associated protein 2; PDE2/4, phosphodiesterases 2A and 4B; AKAP, PKA anchor protein 4; βIPKA, PKA catalytic subunit inhibitor β; PKA, cAMP-dependent protein kinase; CREB, cAMP response element binding protein; CREM/ICER, cAMP response element

modulator/inducible cAMP early repressor; CRI1, CREB binding protein/EP300 inhibitory protein 1; PP1/2A, protein phosphastases 1 and 2A; CRE, cAMP response element.

Fig. 5. Effect of epinephrine on the expression of genes involved in carbohydrate metabolism.

The transcriptionnal pattern suggests that glucose utilization is increased under epinephrine. Filled and open ellipses represent genes down and upregulated, respectively. FBPase-1, fructose-1,6-bisphosphatase; PFK-1, 6-phosphofructo-1-kinase; PFK-2/FBPase-2, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase; PPP1CB, protein phosphatase 1 catalytic subunit beta isoform; PPP1R1A, protein phosphatase 1 regulatory (inhibitor) subunit 1A; branching enzyme, glucan (1,4-alpha-) branching enzyme 1.

Fig. 6. Graphical representation of changes in gene expression and resting metabolic rate during epinephrine infusion.

Upregulated genes with the 20 highest Spearman correlation coefficients are shown. Each line corresponds to one gene identified by UniGene number. Variation in mRNA levels is shown on the x axis. Each dot represents one subject. The colour of the dot corresponds to the extent of the variation in resting metabolic rate (Δresting metabolic rate/resting metabolic rate).

Table 1. Time course of catecholamine plasma concentrations and of various metabolic parameters during the six-hour epinephrine infusion at 0.04 μg.kg^{-1}.min^{-1} in 9 healthy male volunteers

	0h	1h	2h	3h	4h	5h	6h
Epinephine (pg/ml)	23 ± 5			666 ± 55 *			732 ± 75 *
Norepinephrine (pg/ml)	225 ± 32			272 ± 24			218 ± 36
Heart Rate (beat/min)	60 ± 3	62 ± 3	69 ± 4	76 ± 4*	70 ± 5	71 ± 3*	70 ± 4
SBP (mm Hg)	121 ± 3	120 ± 4	126 ± 6	127 ± 4	129 ± 5	127 ± 4	128 ± 4
DBP (mm Hg)	65 ± 1	62 ± 3	58 ± 4	63 ± 3	61 ± 3	59 ± 2	62 ± 2
RMR (J/min/kg LBM)	90.9 ± 3.2	107.4 ± 3.6 *	103.7 ± 3.5 *	107.6 ± 3.2*	106.3 ± 3.0*	108.0 ± 3.4*	108.8 ± 3.7 *
RQ	0.85 ± 0.01	0.81 ± 0.01	0.84 ± 0.01	0.85 ± 0.02	0.85± 0.01	0.85 ± 0.02	0.86 ± 0.02
Glycerol (μmol/l)	58.4 ± 5.9	109.9 ± 6.1 *	98.9 ± 5.6 *	103.5 + 4.1 *	100.5 + 5.3 *	102.7 + 4.1 *	110.6 + 5 *
NEFA (μmol/l)	313 ± 67	840 ± 87 *	643 ± 76 *,§	589 ± 71 *,§	560 ± 78 *,§	551 ± 70 *,§	530 ± 66 *,§
Glucose (mmol/l)	4.90 + 0.14	7.34 + 0.21 *	7.59 + 0.21 *	7.29 + 0.12*	6.81 + 0.15 *,§§	6.82 + 0.15 *,§§	6.61 + 0.16 *,§§
Insulin (mIU/l)	1.62 + 0.28	3.88 + 0.56 *	4.45 + 0.56 *	3.15 + 0.45	2.71 + 0.47 $^{¥}$	2.50 + 0.39 $^{¥}$	2.57 + 0.44

Values are means ± SEM. One way analysis of variance for repeated measures was used for comparisons of the parameters before and during the epinephrine infusion with time as the factor of analysis, followed by a Bonferroni-Dunnett post hoc test with baseline values as controls. * P≤ 0.01 vs 0h; § P ≤ 0.01 vs. 1h (NEFA); §§ P ≤ 0.01 vs. 1h, 2h and 3h (glucose), $^{¥}$ P ≤ 0.01 vs. 2h (insulin). SBP, systolic blood pressure; DBP, diastolic blood pressure; RMR, resting metabolic rate; RQ, respiratory quotient; NEFA, non esterified fatty acids.

Table 2. Comparison of fold changes in mRNA levels determined using microarray and reverse transcription-real time PCR

UniGene n°	Protein name	Category	Microarrays	RT-qPCR
Hs.195471	**6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3**	**Carbohydrate metabolism**	**3.77**	**5.75***
Hs.167382	**Atrial natriuretic peptide receptor A**	**Signal transduction**	**1.55**	**1.61***
Hs.101337	Uncoupling protein 3	Anion transport	1.82	1.51
Hs.9305	**Apelin receptor**	**Signal transduction**	**1.54**	**1.44***
Hs.359682	Calpastatin	Protein metabolism	1.13	1.6
Hs.80658	Uncoupling protein 2	Anion transport	1.05	1
Hs.198468	Peroxisome proliferator activated receptor γ coactivator 1	Energy metabolism	1.04	0.96
Hs.21537	**Protein phosphatase 1, catalytic subunit, β isoform**	**Signal transduction**	**0.57**	**0.53****
Hs.75613	**Fatty acid transporter CD36**	**Lipid metabolism**	**0.63**	**0.74***
Hs.79158	**Medium chain acyl Coenzyme A dehydrogenase**	**Lipid metabolism**	**0.67**	**0.63****
Hs.111779	**Secreted protein acidic and rich in cysteine**	**Tissue remodeling**	**0.71**	**0.79**
Hs.169248	Cytochrome c	Energy metabolism	0.78	0.54
Hs.118836	Myoglobin	Oxygen transport	0.82	0.86
Hs.149155	Voltage-dependent anion channel 1 / porin 31 HL	Anion transport	0.84	0.78*

Fold changes are shown as means of normalized microarray data obtained with amplified RNA and of reverse transcription-real time PCR (RT-qPCR) data obtained with total RNA (n=9). Statistically differential genes sorted by SAM are shown in bold. RT-qPCR data were compared using paired t test (*, $p<0.05$; **, $p<0.01$).

Table 3. Target genes containing cAMP-responsive elements

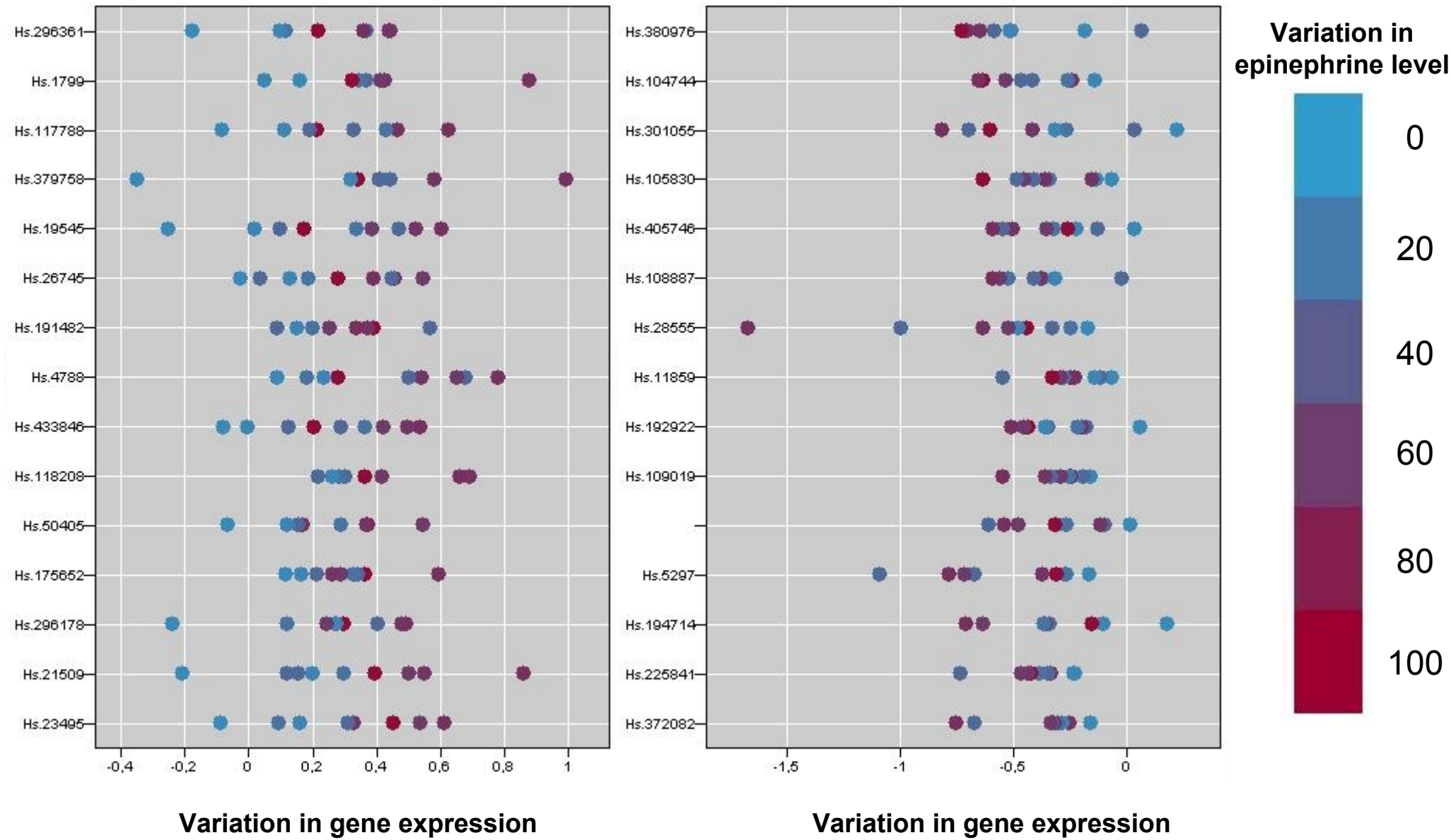
Unigene number	Name	Fold change
Hs.79194	cAMP responsive element binding protein 1 (CREB1)	1.52
Hs.95972	melanoma-associated ME20 antigen	1.48
Hs.93728	pre-B-cell leukemia transcription factor 2 (PBX2)	1.45
Hs.172216	chromogranin A	1.40
Hs.171695	dual specificity phosphatase 1	1.39
Hs.170087	aryl hydrocarbon receptor	1.38
Hs.171596	ephrin type-A receptor 2 precursor (EphA2)	1.32
Hs.58169	highly expressed in cancer, rich in leucine heptad repeats (HEC1)	1.32
Hs.89394	POU domain class 1 transcription factor 1 (Pit1)	1.30
Hs.155924	cAMP responsive element modulator (CREM) / inducible cAMP early repressor (ICER)	1.27
Hs.86859	growth factor receptor-bound protein 7	1.23
Hs.270833	amphiregulin	1.21

Table 4. Genes participating in carbohydrate metabolism regulated by epinephrine in human skeletal muscle

UniGene n°	Protein name	Fold Change
Hs.195471	**6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3**	**3.77**
Hs.69747	**fucosyltransferase 1**	**1.59**
Hs.239106	**solute carrier family 3 , member 1**	**1.52**
Hs.77813	**sphingomyelin phosphodiesterase 1, acid lysosomal**	**1.46**
Hs.119403	**hexosaminidase A (alpha polypeptide)**	**1.45**
Hs.171945	**phospholipase A2 receptor 1, 180kDa**	**1.44**
Hs.1023	**pyruvate dehydrogenase (lipoamide) alpha 1**	**1.43**
Hs.7594	**solute carrier family 2 (facilitated glucose transporter), member 3**	**1.42**
Hs.75658	**phosphorylase, glycogen; brain**	**1.42**
Hs.167584	**solute carrier family 2 (facilitated glucose transporter), member 2**	**1.41**
Hs.76873	**hyaluronoglucosaminidase 2**	**1.38**
Hs.54470	**ATP-binding cassette, sub-family C (CFTR/MRP), member 8**	**1.37**
Hs.133083	**dihydrodiol dehydrogenase (dimeric)**	**1.36**
Hs.92261	**pyruvate dehydrogenase kinase, isoenzyme 2**	**1.34**
Hs.95734	**uridine monophosphate kinase**	**1.34**
Hs.118721	**sialidase 1 (lysosomal sialidase)**	**1.33**
Hs.21899	**solute carrier family 35 (UDP-galactose transporter), member 2**	**1.29**
Hs.78802	**glycogen synthase kinase 3 beta**	**1.27**
Hs.173824	**thymine-DNA glycosylase**	**1.26**
Hs.115945	**mannosidase, beta A, lysosomal**	**1.24**
Hs.98008	**glycerol kinase 2**	**1.22**
Hs.324784	**glutamate decarboxylase 1 (brain, 67kDa)**	**1.20**
Hs.150926	**fucose-1-phosphate guanylyltransferase**	**1.20**
Hs.22983	**UDP-glucose ceramide glucosyltransferase-like 2**	**1.15**
Hs.81897	KIAA1128 protein	0.81
Hs.76780	protein phosphatase 1, regulatory (inhibitor) subunit 1A	0.66
Hs.77837	UDP-glucose pyrophosphorylase 2	0.66
Hs.1691	glucan (1,4-alpha-), branching enzyme 1	0.66
Hs.78060	phosphorylase kinase, beta	0.60
Hs.21537	protein phosphatase 1, catalytic subunit, beta isoform	0.57

Genes relevant for carbohydrate metabolism were searched within the Gene Ontology database (GO:0005975, biological process level 4). Upregulated genes are shown in bold.

Fig. 1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Hs.1799
Hs.117788
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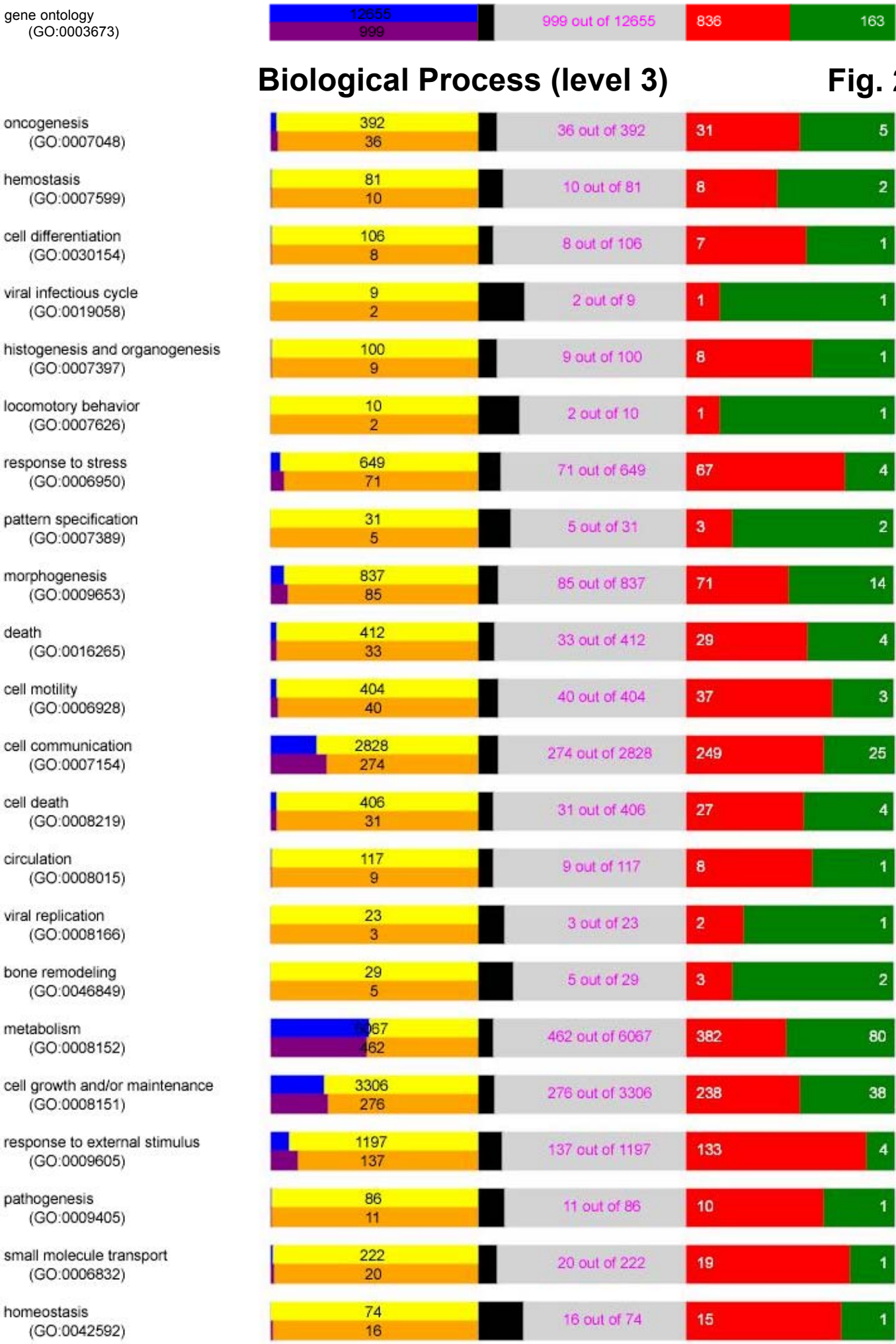
Hs.19545
Hs.26745
Hs.191482
Hs.4788
Hs.433846
Hs.118208
Hs.50405
Hs.175652
Hs.296178
Hs.21509
Hs.23495
-0,4
-0,2
0
0,2
0,4
0,6
0,8
1
Variation in gene expression
Hs.380976
Hs.104744
Hs.301055
Hs.105830
Hs.405746
Hs.108887
Hs.28555
Hs.11859
Hs.192922
Hs.109019
Hs.5297
Hs.194714
Hs.225841
Hs.372082
-1,5
-1
-0,5
0
Variation in gene expression
Variation in epinephrine level
0
20
40
60
80
100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(GO:0003673)
12655
999
999 out of 12655
836
163
Biological Process (level 3)
Fig. 2
oncogenesis
(GO:0007048)
392
36
36 out of 392
31
5
hemostasis
(GO:0007599)
81
10
10 out of 81
8
2
cell differentiation
(GO:0030154)
106
8
8 out of 106
7
1
viral infectious cycle
(GO:0019058)
9
2
2 out of 9
1
1
histogenesis and organogenesis
(GO:0007397)
100
9
9 out of 100
8
1
locomotory behavior
(GO:0007626)
10
2
2 out of 10
1
1
response to stress
(GO:0006950)
649
71
71 out of 649
67
4
pattern specification
(GO:0007389)
31
5
5 out of 31
3
2
morphogenesis
(GO:0009653)
837
85
85 out of 837
71
14
death
(GO:0016265)
412
33
33 out of 412
29
4
cell motility
(GO:0006928)
404
40
40 out of 404
37
3
cell communication
(GO:0007154)
2828
274
274 out of 2828
249
25
cell death
(GO:0008219)
406
31
31 out of 406
27
4
circulation
(GO:0008015)
117
9
9 out of 117
8
1
viral replication
(GO:0008166)
23
3
3 out of 23
2
1
bone remodeling
(GO:0046849)
29
5
5 out of 29
3
2
metabolism
(GO:0008152)
462
462 out of 6067
382
80
cell growth and/or maintenance
(GO:0008151)
3306
276
276 out of 3306
238
38
response to external stimulus
(GO:0009605)
1197
137
137 out of 1197
133
4
pathogenesis
(GO:0009405)
86
11
11 out of 86
10
1
small molecule transport
(GO:0006832)
222
20
20 out of 222
19
1
homeostasis
(GO:0042592)
74
16
16 out of 74
15
1

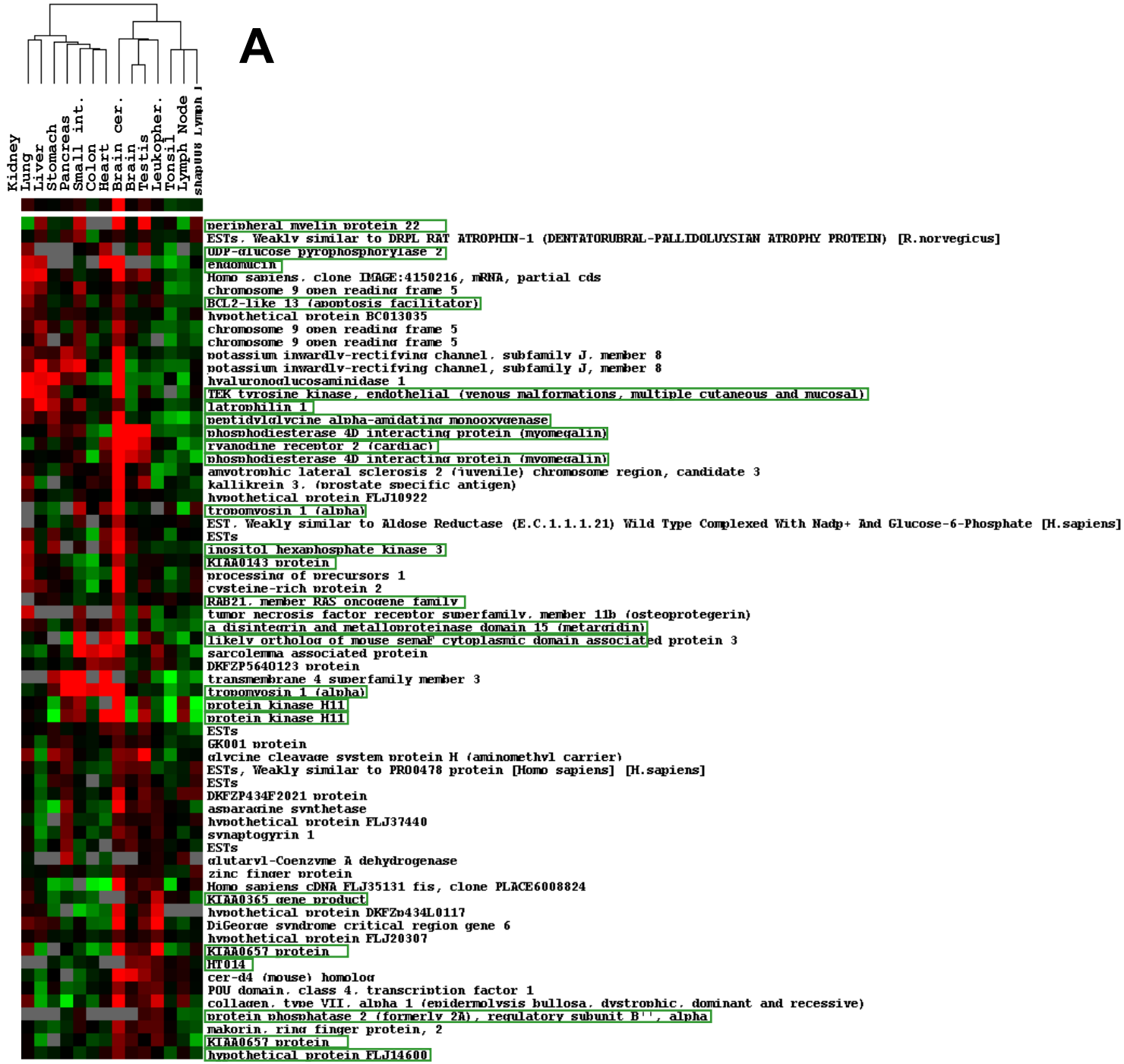
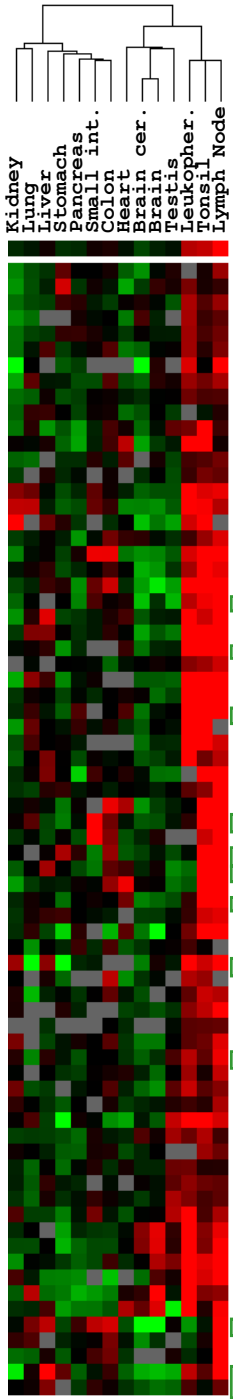

A
Kidney
Lung
Liver
Stomach
Pancreas
Small int.
Colon
Heart
Brain cer.
Brain
Testis
Leukopher.
Tonsil
Lymph Node
peripheral myelin protein 22
ESTs, Weakly similar to DRPL_RAT ATROPHIN-1 (DENTATORUBRAL-PALLIDOLUYSIAN ATROPHY PROTEIN) [R.norvegicus]
UDP-glucose pyrophosphorylase 2
endomucin
Homo sapiens, clone IMAGE:4150216, mRNA, partial cds
chromosome 9 open reading frame 5
BCL2-like 13 (apoptosis facilitator)
hypothetical protein BC013035
chromosome 9 open reading frame 5
chromosome 9 open reading frame 5
potassium inwardly-rectifying channel, subfamily J, member 8
potassium inwardly-rectifying channel, subfamily J, member 8
hyaluronoglucosaminidase 1
TEK tyrosine kinase, endothelial (venous malformations, multiple cutaneous and mucosal)
latrophilin 1
peptidylglycine alpha-amidating monooxygenase
phosphodiesterase 4D interacting protein (myomegalin)
ryanodine receptor 2 (cardiac)
phosphodiesterase 4D interacting protein (myomegalin)
amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 3
kallikrein 3, (prostate specific antigen)
hypothetical protein FLJ10922
tropomyosin 1 (alpha)
EST, Weakly similar to Aldose Reductase (E.C.1.1.1.21) Wild Type Complexed With Nadp+ And Glucose-6-Phosphate [H.sapiens]
ESTs
inositol hexaphosphate kinase 3
KIAA0143 protein
processing of precursors 1
cysteine-rich protein 2
RAB21, member RAS oncogene family
tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)
a disintegrin and metalloproteinase domain 15 (metargidin)
likely ortholog of mouse semaF cytoplasmic domain associated protein 3
sarcolemma associated protein
DKFZP564O123 protein
transmembrane 4 superfamily member 3
tropomyosin 1 (alpha)
protein kinase H11
protein kinase H11
ESTs
GK001 protein
glycine cleavage system protein H (aminomethyl carrier)
ESTs, Weakly similar to PRO0478 protein [Homo sapiens] [H.sapiens]
ESTs
DKFZP434F2021 protein
asparagine synthetase
hypothetical protein FLJ37440
synaptogyrin 1
ESTs
glutaryl-Coenzyme A dehydrogenase
zinc finger protein
Homo sapiens cDNA FLJ35131 fis, clone PLACE6008824
KIAA0365 gene product
hypothetical protein DKFZp434L0117
DiGeorge syndrome critical region gene 6
hypothetical protein FLJ20307
KIAA0657 protein
HT014
cer-d4 (mouse) homolog
POU domain, class 4, transcription factor 1
collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
protein phosphatase 2 (formerly 2A), regulatory subunit B'', alpha
makorin, ring finger protein, 2
KIAA0657 protein
hypothetical protein FLJ14600

Fig. 3



B

Fig. 3

Werner syndrome
myosin IXB
synaptotagmin I
KIAA0087 gene product
special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)
osteoclast stimulating factor 1
killer cell lectin-like receptor subfamily C, member 1
Human PVT-IGLC fusion protein mRNA, 5' end
guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 1
zinc finger protein 24 (KOX 17)
nudix (nucleoside diphosphate linked moiety X)-type motif 1
signal transducer and activator of transcription 4
chromosome 9 open reading frame 5
phosphatidylserine synthase 1
chemokine (C-C motif) ligand 5
butyrophilin, subfamily 3, member A2
phospholipase C, gamma 2 (phosphatidylinositol-specific)
mitogen-activated protein kinase kinase kinase kinase 2
interferon stimulated gene 20kDa
interferon, gamma-inducible protein 16
interleukin 2 receptor, gamma (severe combined immunodeficiency)
ribonuclease, RNase A family, k6
protease, serine, 2 (trypsin 2)
Src-like-adaptor
selectin L (lymphocyte adhesion molecule 1)
NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa
protein tyrosine phosphatase, receptor type, C
runt-related transcription factor 3
CD3G antigen, gamma polypeptide (TiT3 complex)
CD5 antigen (p56-62)
a disintegrin and metalloproteinase domain 8
leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 4
pleckstrin
splicing factor, arginine/serine-rich 2, interacting protein
matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
complement component (3d/Epstein Barr virus) receptor 2
diacylglycerol kinase, alpha 80kDa
paired box gene 5 (B-cell lineage specific activator protein)
chemokine (C-C motif) ligand 19
Duffy blood group
CD79B antigen (immunoglobulin-associated beta)
mitogen-activated protein kinase kinase kinase 14
ESTs
ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens]
chemokine (C-C motif) ligand 4
c-src tyrosine kinase
highly expressed in cancer, rich in leucine heptad repeats
N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1
EST
cell growth regulatory with ring finger domain
src family associated phosphoprotein 1
TXK tyrosine kinase
CHK1 checkpoint homolog (S. pombe)
kinesin family member 14
PAS domain containing serine/threonine kinase
Homo sapiens cDNA FLJ39225 fis, clone OCBBF2007224, highly similar to Homo sapiens candidate tumor suppressor protein DICE1 mRNA
myosin, heavy polypeptide 11, smooth muscle
primase, polypeptide 1, 49kDa
ring finger protein 4
hypothetical protein FLJ20552
myosin regulatory light chain interacting protein
FYN oncogene related to SRC, FGR, YES
FYN oncogene related to SRC, FGR, YES
KIAA0449 protein
sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D
potassium voltage-gated channel, shaker-related subfamily, beta member 2
endothelial differentiation, sphingolipid G-protein-coupled receptor, 1
chemokine (C-C motif) ligand 15
ESTs
multimerin
CD14 antigen
CD14 antigen

Epinephrine

Fig. 4

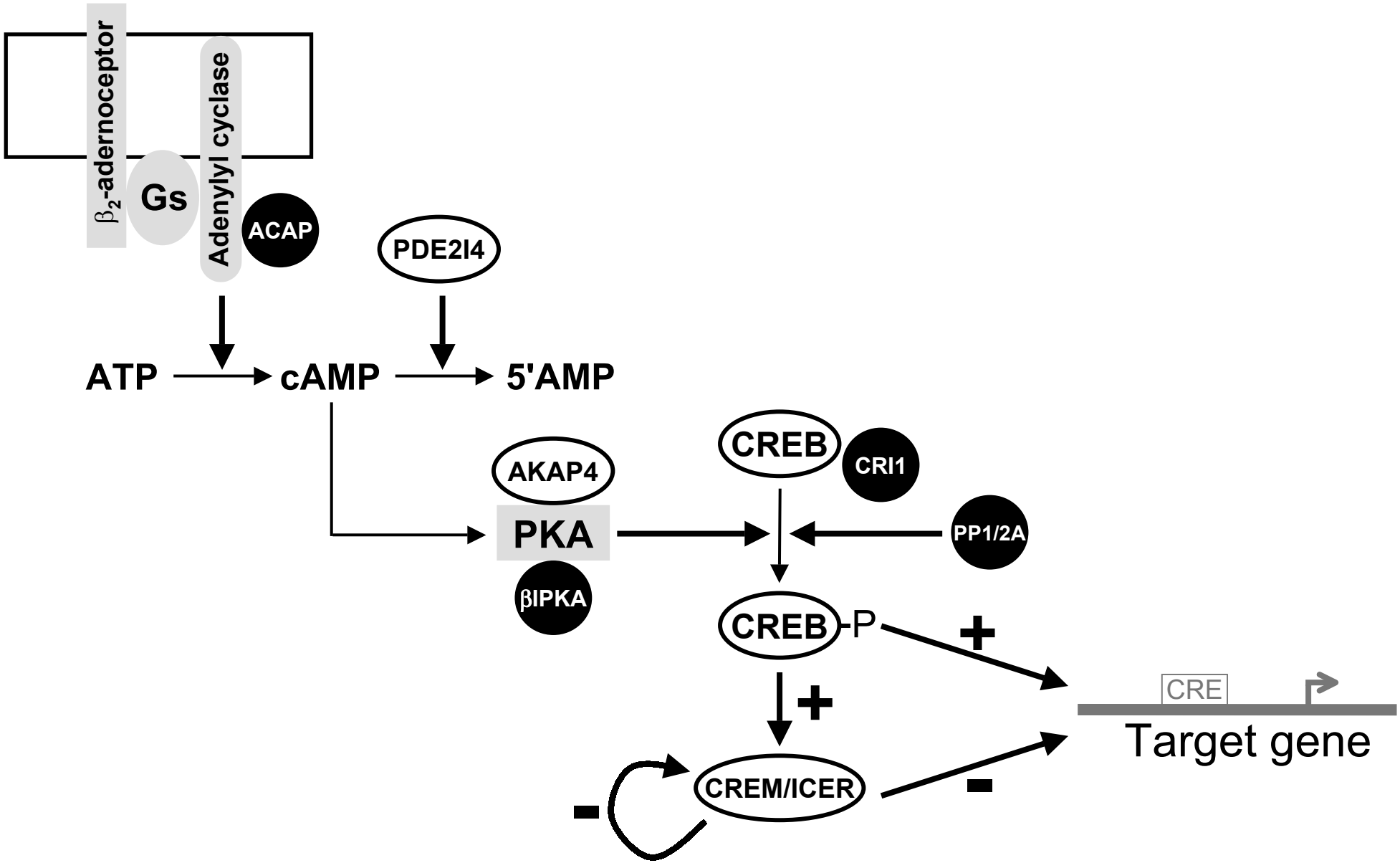


Fig. 5

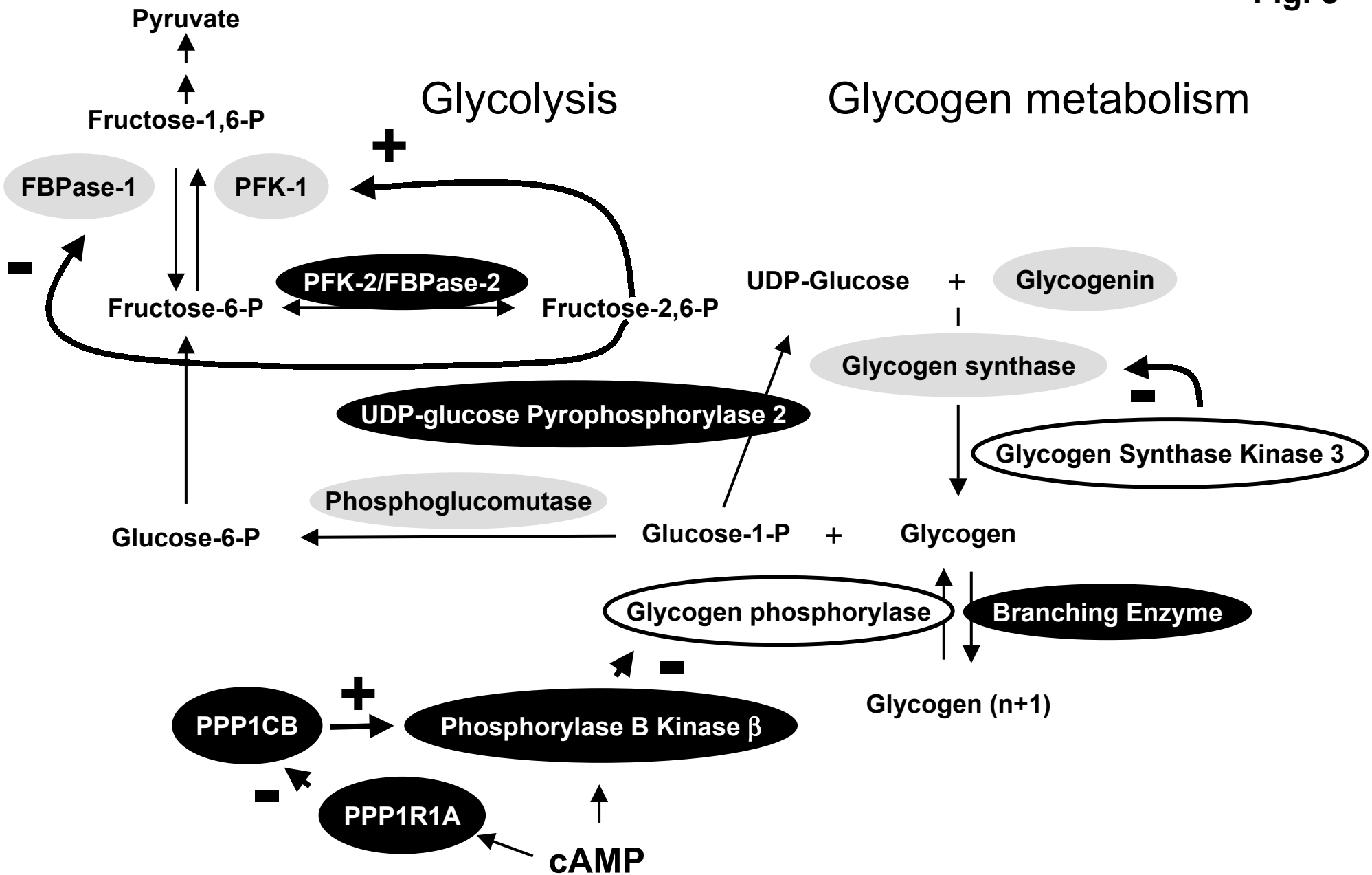
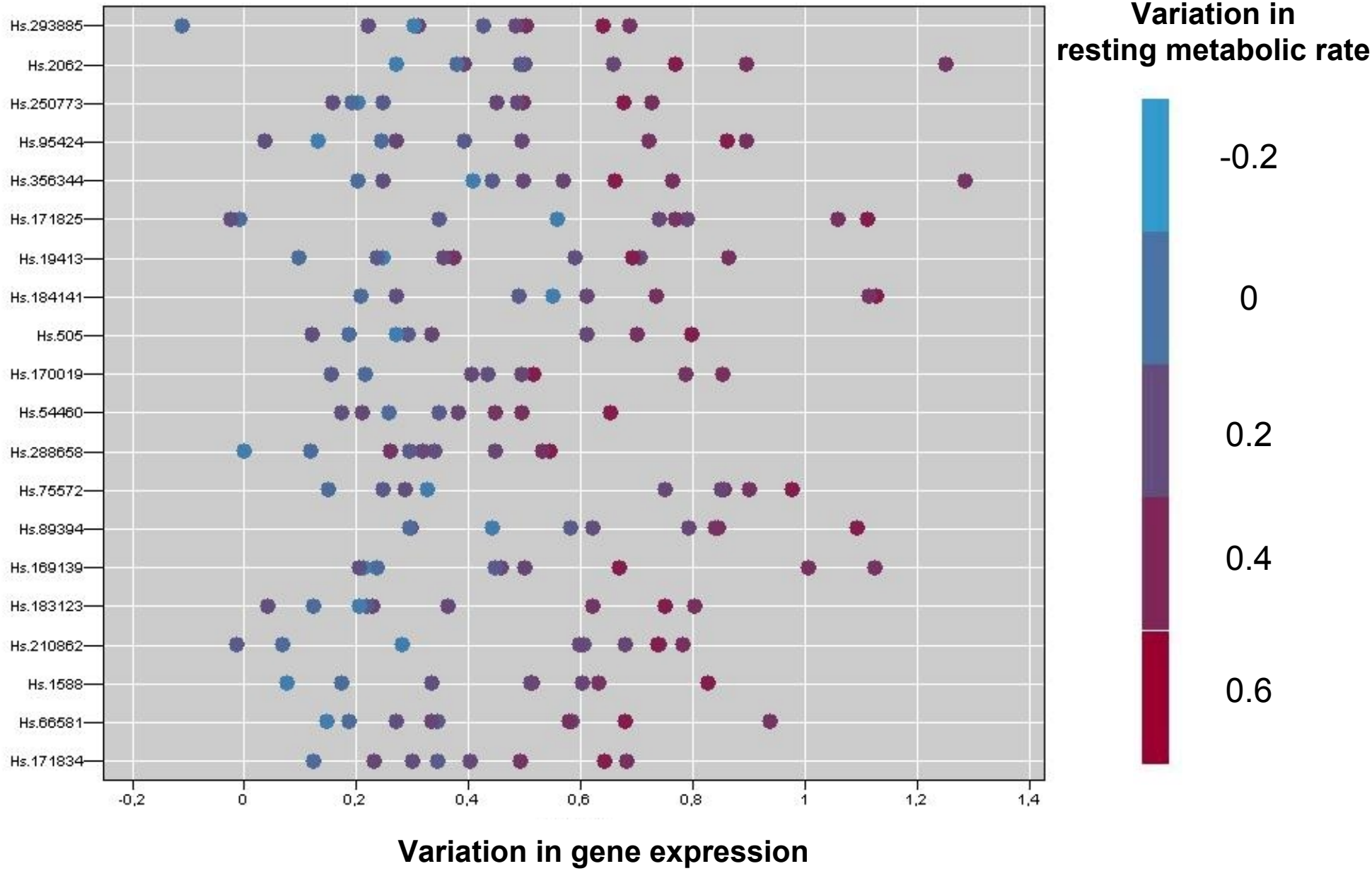

Pyruvate
Fructose-1,6-P
Glycolysis
Glycogen metabolism
FBPase-1
PFK-1
+
-
Fructose-6-P
PFK-2/FBPase-2
Fructose-2,6-P
UDP-Glucose
+
Glycogenin
Glycogen synthase
-
UDP-glucose Pyrophosphorylase 2
Glycogen Synthase Kinase 3
Phosphoglucomutase
Glucose-6-P
Glucose-1-P
+
Glycogen
Glycogen phosphorylase
Branching Enzyme
Glycogen (n+1)
-
+
PPP1CB
Phosphorylase B Kinase β
-
PPP1R1A
cAMP

Fig. 6


Hs.293885
Hs.2062
Hs.250773
Hs.95424
Hs.356344
Hs.171825
Hs.19413
Hs.184141
Hs.505
Hs.170019
Hs.54460
Hs.288658
Hs.75572
Hs.89394
Hs.169139
Hs.183123
Hs.210862
Hs.1588
Hs.66581
Hs.171834
-0,2
0
0,2
0,4
0,6
0,8
1
1,2
1,4
Variation in gene expression
Variation in resting metabolic rate
-0.2
0
0.2
0.4
0.6