The role of energy & fatty acid metabolism in obesity and insulin resistance

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Reanalysis of mGWAS results and in vitro validation show that lactate dehydrogenase interacts with branched-chain amino acid metabolism

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Abstract

The assignment of causative genes to noncoding variants identified in Genome Wide Association Studies (GWASs) is challenging. We show how combination of knowledge from gene and pathway databases and chromatin interaction data leads to reinterpretation of published quantitative trait loci for blood metabolites. We describe a previously unidentified link between the rs2403254 locus, which is associated with the ratio of 3-methyl-2-oxobutanoate and alpha-hydroxyisovalerate levels, and the distal *LDHA* gene. We confirmed that lactate dehydrogenase can catalyze the conversion between these metabolites *in vitro*, suggesting that it plays a role in branched-chain amino acid metabolism. Examining datasets from the ENCODE project we found evidence that the locus and *LDHA* promoter physically interact, showing that *LDHA* expression is likely under control of distal regulatory elements. Importantly, this discovery demonstrates that bioinformatic workflows for data integration can play a vital role in the interpretation of GWAS results.

Introduction

Due to recent advances in metabolomics technology and decreasing costs, Genome Wide Association Studies have now been performed on a wide range of metabolites [1–4]. These studies have provided new insights into how biochemical pathways are affected by genetic polymorphisms and have increased our understanding of the pathogenesis of metabolic disease [3,5]. Since variation in metabolite levels is often linked to changes in enzyme or transporter activity, functional annotation of the loci that are located in or close to enzyme coding genes has been straightforward. However, for a substantial part of the loci identified in mGWAS no obvious link between the metabolite and proximal enzyme coding genes exists, and the association with the phenotype is much harder to explain.

We developed an automated workflow for mapping the results of GWASs on pathway databases to assist in their interpretation. We applied the workflow on the 37 loci that have been reported by Suhre *et al.* [3] and were able to provide a new functional annotation of the rs2403254 (chr11.hg19:g.18325146C>T) single nucleotide polymorphism (SNP) which associates with the ratio of 3-methyl-2-oxobutanoate and alpha-hydroxyisovalerate levels in the blood. Reanalysis of this locus uncovered a functional link to the gene coding for the lactate dehydrogenase (LDH) A enzyme and *in vitro* analysis confirmed that LDH could convert 3-methyl-2-oxobutanoate into alpha-hydroxyisovalerate. In addition, we found a physical association between the rs2403254 locus and the *LDHA* promoter region in the chromatin interaction data from the ENCODE project [6–8]. Combined, our data suggest that LDH interacts with branched-chain amino acid metabolism and is affected by genetic variation at a distal locus.

Materials and methods

Automated annotation of GWAS results

In order to facilitate the manual process of assigning a gene to each locus, we developed an automated workflow *in house* to generate reports containing the associated protein, enzyme, metabolic reaction, pathway, and disease phenotypes of each gene within a distance of 500 kb of the locus. In detail, the reports created by our workflow were based on the dbSNP[9], NCBI-Gene (http://www.ncbi.nlm.nih.gov/gene), ConsensusPathDB[10], UniProtKB[11], OMIM[12], Gene Ontology[13], TCDB[14], ExPASy[15] and KEGG database[16]. The databases had been downloaded earlier from the respective ftp servers and have been integrated offline using MATLAB (R2010a, The Mathworks Inc., Natick, MA, USA).

Characterization of rs2403254 locus

Chromatin accessibility and interactions were initially evaluated in the UCSC genome browser (http://genome.ucsc.edu). Subsequently cell-type specific DHS correlations and ChIA-PET (K562 Pol II) data were imported in MATLAB and superimposed on the regional LD data of the rs2403254 locus based on the 1000 Genomes Pilot 1 data of the CEU population (calculated using SNAP[17]). Since the ChIA-PET K562 Pol II data comprised two replicates, we only considered interactions for which the interacting regions overlapped between both replicates. Finally, eQTLs associated with *LDHA* expression as reported in the GTEx (http://www.ncbi.nlm.nih.gov/gtex/GTEX2) and gEUVADIS[18] database were imported and added to the regional LD plot.

Lactate dehydrogenase activity

Enzyme kinetic measurements were done in a 96 well plate in quadruple, based on the protocol of Vassault *et al.* [19]. Briefly, substrates were dissolved in buffer solution (80 mM TrisHCl, 0.20 mM NADH, pH 7.2 at 30°C) to the final concentrations mentioned in figure 2 in 180 µl. Lactate dehydrogenase was added (20 µl) containing $2.03 \bullet 10^{-5}$ mg LDH per well for pyruvate, $9.27 \bullet 10^{-3}$ mg for 3-methyl-2-oxobutanoate, $9.27 \bullet 10^{-2}$ mg for 3-methyl-2-oxopentanoate and $1.63 \bullet 10^{-2}$ mg for 4-methyl-2-oxopentanoate reactions. The decrease of A_{340} nm for NADH was measured every 8 sec for 6 min after which the slope was taken and using a calibration curve for NADH was recalculated as specific activity (U) in µmol NADH min^{-1} mg^{-1} LDH.

Data availability

We have submitted our finding of the link between rs2403254 and *LDHA* to the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar; accession number SCV000211950).

Results

Automated annotation of GWAS results

The automated workflow we developed was used to generate reports for each of the 37 SNPs published by Suhre *et al.* [3], containing the associated protein, enzyme, metabolic reaction, pathway, and disease phenotypes of each gene within a distance of 500 kb of the locus. Inspection of the results showed that for one of the 37 SNPs, rs2403254, there was an alternative candidate gene that provided a more likely explanation of the association. The rs2403254 SNP was associated with alpha-hydroxyisovalerate levels in the blood ($p=1.0 \cdot 10^{-20}$) and showed an even stronger correlation with the ratio of 3-methyl-2-oxobutanoate and alpha-hydroxyisovalerate levels ($p=7.9 \cdot 10^{-28}$). The rs2403254 SNP lies inside the *HPS5* gene and had therefore been assigned to *HPS5* by Suhre and colleagues. However, from inspection of the results from our workflow it followed that a plausible alternative candidate gene in the vicinity of the locus was *LDHA*, which codes for the A-isoform of the lactate dehydrogenase (LDH) enzyme. In addition, from information retrieved from the KEGG database [16] it followed that LDH has a broad substrate specificity and can catalyze the conversion of several keto and hydroxy acids, even though 3-methyl-2-oxobutanoate or alpha-hydroxyisovalerate were not listed as substrates (Supplemental Text S1).

The rs2403254 locus

Closer examination of the locus showed that rs2403254 is located in a large linkage disequilibrium (LD) block, which lies approximately 20kb upstream of *LDHA* (Fig. 1). To investigate the presence of potential long-distance regulatory mechanisms, we first looked at expression Quantitative Trait Loci (eQTLs) in lymphoblastoid cell lines that were associated with *LDHA*. These eQTLs were all located downstream of the LD block and were not in strong LD with rs2403254. In contrast, we found that rs2403254 is an eQTL for *HPS5* ($p=4.4 \cdot 10^{-9}$) and *GTF2H1* ($p=8.4 \cdot 10^{-15}$), most likely because it is in strong LD ($R^2 > 0.8$) with several SNPs that lie close to the transcription start sites of these genes.

Subsequently we explored chromatin interactions and the presence of regulatory elements in the LD block by looking at the DNase I signal data from the ENCODE project [6–8]. Interestingly, several regulatory regions were present within the LD block whose DNase I signal had a strong cross-cell-type correlation with the *LDHA* promoter (Fig. 1; bottom). These potential distal interactions were supported by the Chromatin Interaction Analysis with Paired-End-Tag (ChIA-PET) sequencing data, which showed that there were significant chromatin interactions between the central region of the LD block and the region containing the *LDHA* promoter. Collectively, these data show that long-range regulation of *LDHA* by enhancers that are located more than 20 kbp upstream of its transcription start site is indeed plausible.

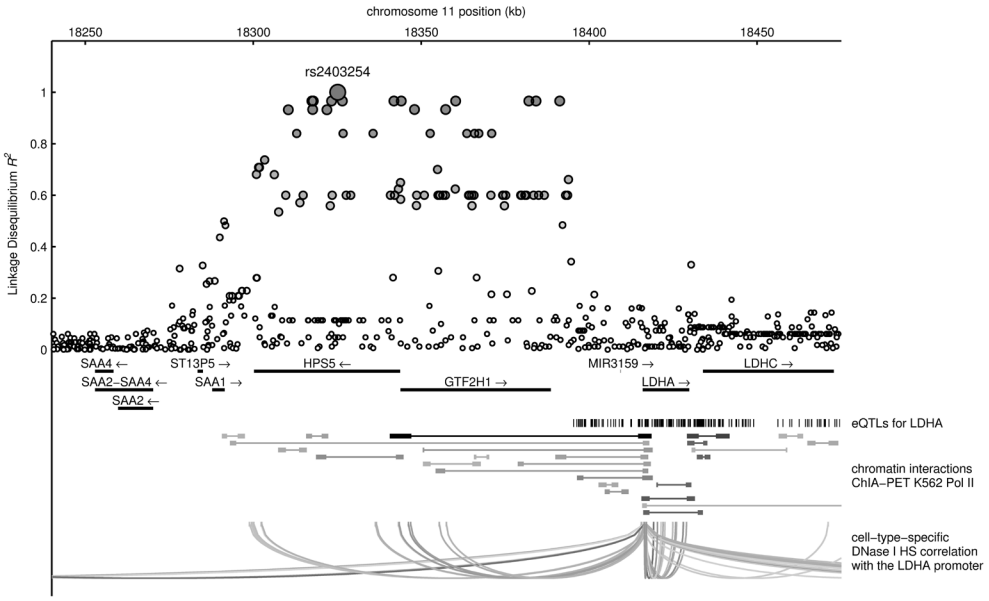

Figure 1: Regional Linkage Disequilibrium (LD) plot of the rs2403254 locus. The rs2403254 locus is located in a region of strong LD spanning over approximately 100kb. Several eQTLs have been identified that associate with LDHA expression, but none lie in LD with rs2403254. In contrast, chromatin interaction ChIA-PET data and cell type specific DNase I hypersensitive site correlations show that there are several chromatin interactions between the LDHA promoter and regulatory elements within the LD block.

Experimental validation

Subsequently, we investigated whether LDH could catalyze the conversion between branched-chain alpha-keto and hydroxy acids. In previous studies LDH had already been shown to catalyze a broad range of substrates [20, 21], but because of the extremely low rates with which branched-chain alpha-keto and hydroxy acids were converted it was unclear whether this reaction was mediated by LDH or another dehydrogenase [22, 23]. We therefore validated our finding by assaying the activity of LDH in the presence of NADH and the transaminated branched-chain keto acid products 3-methyl-2-oxobutanoate (valine), 4-methyl-2-oxopentanoate (leucine), 3-methyl-2-oxopentanoate (isoleucine) and pyruvate for a range of different substrate concentrations (Fig. 2). Results show that LDH was indeed able to convert 3-methyl-2-oxobutanoate, but it had a specificity constant k_{cat}/K_m that was approximately 3000 times lower than with pyruvate as substrate (Table 1). In comparison, the k_{cat}/K_m of the other two branched-chain alpha-keto acids was around 10 times lower than with 3-methyl-2-oxobutanoate as substrate. Most probably 3-methyl-2-oxobutanoate is converted more efficiently by LDH because it is a smaller molecule, having a carbon chain of 4 atoms, whereas 3- and 4-methyl-2-oxopentanoate have a carbon chain of 5 atoms. Interestingly, the K_m value for pyruvate and 3-methyl-2-oxopentanoate were very similar, while for the other two branched-chain alpha-keto acids it was 3 to 4 times larger.

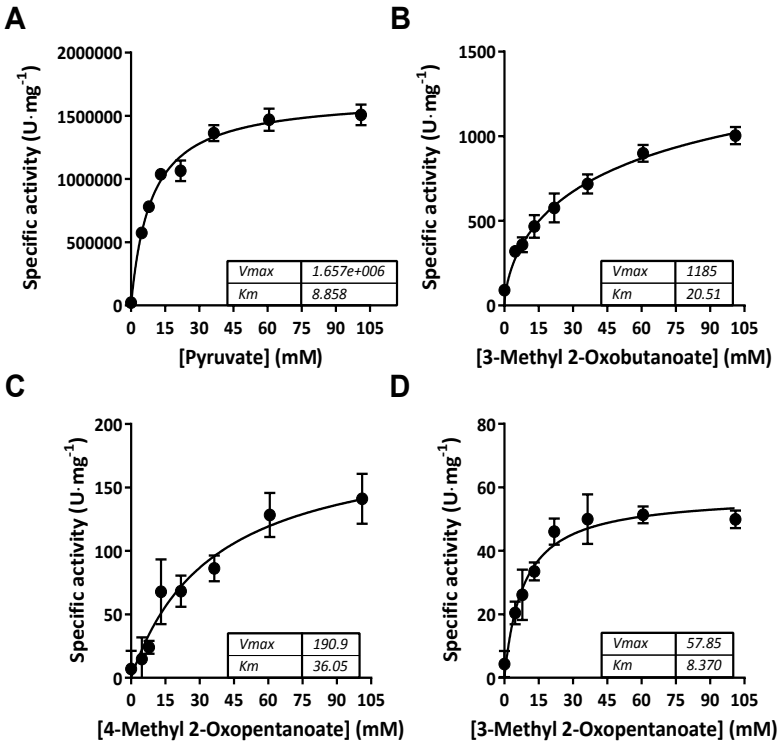

Figure 2: Enzyme kinetics of rabbit muscle lactate dehydrogenase. Specific activity in µmol NADH min^{-1} mg^{-1} LDH of rabbit muscle lactate dehydrogenase for A) pyruvate, B) 3-methyl-2-oxobutanoate, C) 4-methyl-2-oxopentanoate and D) 3-methyl-2-oxopentanoate. Assay conditions were 30°C, pH 7.2, initial [NADH]=0.20 mM.

*Table 1: Kinetic parameters of rabbit muscle lactate dehydrogenase. K_m and k_{cat} are Michaelis-Menten parameters ($v = k_{cat}[E][S] / (K_m+[S])$), while h, $K_{1/2}$ and k_{cat}' are allosteric sigmoidal parameters ($v = k_{cat}'[E][S]^h / (K_{1/2}{}^h+[S]^h)$). *p<0,05 for allosteric sigmoidal kinetics compared to Michaelis-Menten kinetics.*

Substrate	**k_{cat}** [µmol min^{-1} mg^{-1}]	**k_{cat}/K_m** [ml^{-1} min^{-1} mg^{-1}]	**h**	**$K_{1/2}$** [mM]	**k_{cat}'** [µmol min^{-1} mg^{-1}]
pyruvate	$1.66 \cdot 10^6$	$1.87 \cdot 10^5$			
3-methyl-2-oxobutanoate	$1.19 \cdot 10^3$(*)	58.0	0.65	66.6	$1.80 \cdot 10^3$
3-methyl-2-oxopentanoate	57.9	6.9			
4-methyl-2-oxopentanoate	190.9	5.3			

Discussion

The discovery of the functional link between the rs2403254 locus and the *LDHA* gene has been made possible by the automated workflow we developed for annotating GWAS results, which allowed us to examine the set of loci reported by Suhre *et al.* [3] in both a quick and thorough manner. In recent years there has been an increasing interest in bioinformatics tools for the analysis, interpretation and integration of results from GWASs [24, 25]. Suhre and colleagues have employed the tool GRAIL from the Broad Institute [26] in their study, which uses textual relationships between genes to prioritize candidate genes for a given locus. Nonetheless, using this method the authors did not identify *LDHA* as a plausible candidate for the rs2403254 locus, most likely because alpha-hydroxyisovalerate is currently not present in pathway databases and its link to LDH has only been scarcely described in the literature. In fact, the locus was replicated in a recent meta-analysis on the same metabolomics platform [27], but *HPS5* was still proposed as candidate gene.

In contrast, with our approach we focused on integrating the knowledge present in several databases in order to produce succinct SNP reports containing relevant information about all neighboring genes. In the case of the rs2403254 locus the SNP report showed that *LDHA* was the closest gene with a metabolic function and that LDH was documented in the KEGG pathway database as an enzyme that can catalyze multiple reactions. Subsequent investigation of the chemical structure of alpha-hydroxyisovalerate suggested that - in principle - its conversion could be catalyzed by LDH. This hypothesis was further reinforced by the observation that the same locus had a stronger association with the ratio of alpha-hydroxyisovalerate and 3-methyl-2-oxobutanoate levels and that alpha-hydroxyisovalerate is the product of 3-methyl-2-oxobutanoate after reduction of the alpha-carbonyl group.

Our results suggest that there is a functional link between *LDHA* and alpha-hydroxyisovalerate levels and, more specifically, that LDH can compensate for large build-ups of branched-chain alpha-keto acids under hypoxic conditions. In fact, the first step of branched-chain amino acid catabolism involves the transamination of the amino group, which changes the oxidation level of the adjacent carbon atom. Under anaerobic conditions the redox balance needs to be restored, which can be achieved through LDH by converting the alpha-keto acid into an alpha-hydroxycarboxylic acid (Fig. 2). This process has been observed in babies who suffered from asphyxia during birth, where elevated levels of alpha-hydroxyisovalerate were found in the urine [28]. Interestingly, also infants that suffer from Maple syrup urine disease, which is a defect in any of the genes coding for the components of the BCKDH enzyme complex, have elevated levels of alpha-hydroxyisovalerate in the urine [29]. This can be explained by the fact that a blockage in the second step of the branched-chain amino acid degradation pathway causes a build-up of keto acid intermediates, which are then partly converted to hydroxycarboxylic acids via LDH (Fig. 3).

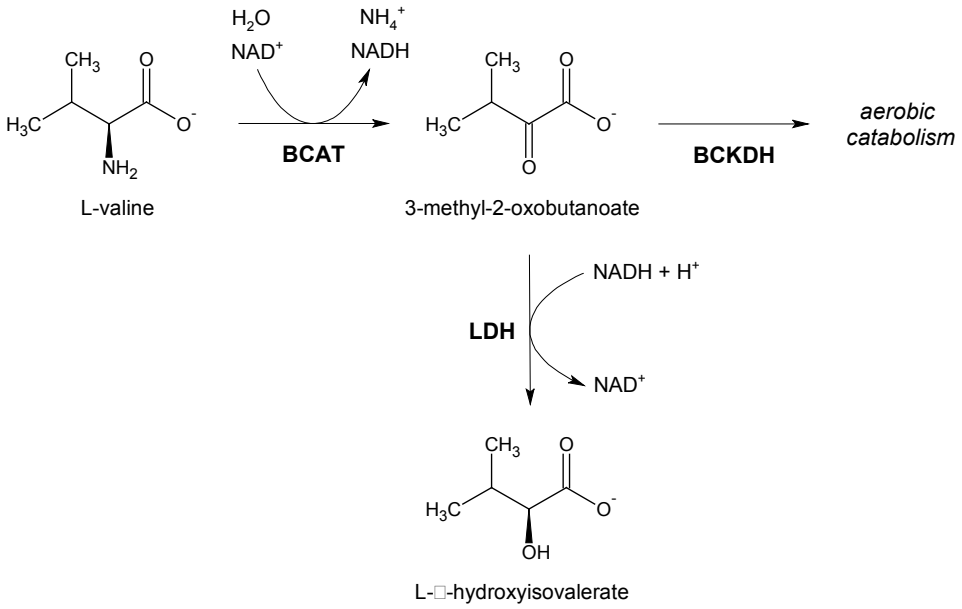

Figure 3: Visualization of the valine degradation pathway and the interaction with lactate dehydrogenase. Abbreviations of enzyme names are shown in boldface (BCAT: branched-chain aminotransferase; BCKDH: branched-chain alpha-keto acid dehydrogenase; LDH: lactate dehydrogenase). LDH can convert 3-methyl-2-oxobutanoate, which is the product of valine after transamination, into alpha-hydroxyisovalerate to balance the redox potential under hypoxic conditions. Under aerobic conditions 3-methyl-2-oxobutanoate can be further degraded by the enzymes of branched-chain amino acid catabolism. In contrast, alpha-hydroxyisovalerate cannot be metabolized any further and is excreted in urine.

Interestingly, in the meta-analysis of Shin *et al.* [27] rs2403254 was found to have the strongest association with the ratio of 3-(4-hydroxyphenyl)lactate and alpha-hydroxyisovalerate levels. 3-(4-hydroxyphenyl)lactate is the product of 4-hydroxyphenylpyruvate after reduction of the alpha-carbonyl group, which is the product of tyrosine after transamination. Given their structural differences it is unlikely that 3-(4-hydroxyphenyl)lactate and alpha-hydroxyisovalerate can be converted into one another in one or two enzymatic steps. The association with rs2403254 is therefore probably due to other mechanisms, such as co-regulation of aromatic and branched-chain amino acid metabolism.

Importantly, our finding does not preclude effects of rs2403254 on *HPS5* or other genes. In fact, in our analysis we found that rs2403254 is an expression QTL for *HPS5* and *GTF2H1*, which shows that multiple genes can be affected by a single SNP. However, neither of these genes provide a biochemical explanation of the phenotype, namely, why rs2403254 is associated with the ratio of 3-methyl-2-oxobutanoate and alpha-hydroxyisovalerate levels. The absence of eQTLs for *LDHA* in the databases that we enquired seems to confirm that distal eQTLs are more difficult to identify and demonstrates the additional value of chromatin interaction data to establish SNP-gene interactions.

In conclusion, we have uncovered a novel functional link between lactate dehydrogenase and the branched-chain keto acid intermediate of valine metabolism by reanalyzing published mGWAS results using automated workflows for integrating information from pathway databases.

Acknowledgements

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Supplemental data

Text S1: SNP report of the rs2403254 locus for LDHA. Similar gene-reports were generated for all genes within a 500 kbp distance of rs2403254, as retrieved from NCBI-Gene, ConsensusPathDB, UniProtKB, OMIM, Gene Ontology, TCDB, ExPASy and KEGG database.

SNP rs2403254 Chr 11 18325146

Distance to locus: -90790 LD R2 of rs2403254 with intragenic SNP rs3758683: 0.11936
Gene: **3939** (LDHA) Chr 11 18415936-18429765 (+)

23516535 the role played by ERRalpha in the regulation of lactate dehydrogenases A and B

23404405 Lactate dehydrogenase A is overexpressed in pancreatic cancer.

23266049 Data indicate that serum lactic dehydrogenase (S-LDH) appears to be a significant independent prognostic index in patients with metastatic nasopharyngeal carcinoma (NPC).

23184277 Increased LDH5 expression is associated with lymph node metastasis in oral squamous cell carcinoma.

23166385 Elevated lactate dehydrogenase level is associated with recurrent or refractory aggressive lymphoma.

22961700 LDHA plays an important role in the progression of esophageal squamous cell carcinoma by modulating cell growth

22948140 Cells expressing either PDK1 or LDHA maintained a lower mitochondrial membrane potential and decreased reactive oxygen species production with or without exposure to toxins.

22923663 Lactic acid induces myofibroblast differentiation via pH-dependent activation of transforming growth factor beta.

22897481 Studies indicate the mechanisms by which lactate dehydrogenase A (LDHA) promotes tumor growth and metastasis.

22593701 Data suggest that serum lactate dehydrogenase (LDH) kinetics might reflect disease behaviour in extracranial metastatic and primary sites without need for comprehensive imaging studies and is a quite inexpensive diagnostic test.

22429998 We demonstrate that LDH-A reduction can suppress the tumorigenicity of intestinal-type gastric cancer (ITGC) cells by downregulating Oct4 both in vitro and in vivo.

22360420 A protein encoded by this locus was found to be differentially expressed in postmortem brains from patients with atypical frontotemporal lobar degeneration.

22127970 Case Report: describe an elderly patient with physical therapy-induced rhabdomyolysis complicated by acute kidney injury associated with reduced skeletal muscle LDH-A activity.

21969607 LDH-A is tyrosine phosphorylated and activated by FGFR1 in cancer cells.

21858537 Results show that S-100B, MIA and LDH levels were significantly higher in patients with advanced melanoma than in disease-free patients or healthy controls.

21632858 Serum LDH and tissue LDH5 levels are complementary features that help to characterize the activity of LDH in colorectal cancer and have a potent value in predicting response to chemotherapy.

21452021 LDH-A reduction resulted in an inhibited cancer cell proliferation, elevated intracellular oxidative stress, and induction of mitochondrial pathway apoptosis.

21249322 High LDH is associated with M1b prostate cancer.

20951115 Presented are QM/MM calculations that show differences in geometries of active sites of M(4) and H(4) isoforms of human LDH ligated with oxamate, pyruvate or L-lactate.

20828817 High lactate dehydrogenase is associated with acute adult T-cell leukemia/ lymphoma.

20385008 LDH5 is overexpressed in non-small cell lung cancer and could serve as a marker for malignancy. LDH5 correlates positively with the prognostic marker transketolase like 1 protein.

19923867 LDH5 is highly expressed in squamous cell head and neck cancer and is linked with local relapse, survival and distant metastasis.

19847924 Observational study and genome-wide association study of gene-disease association. (HuGE Navigator)

19838163 Correlation of LDH-5 expression with clinicopathological factors and with the expression of Bcl-2, Bcl-XL, Mcl-1 and GRP78 was examined in pigmented lesions, including nevi and melanoma at different stages of progression

19668225 ErbB2 promotes glycolysis at least partially through the HSF1-mediated upregulation of LDH-A.

19276158 LDH-A knockdown in the background of FH knockdown results in significant reduction in tumor growth in a xenograft mouse model.

19021062 LDH5 is highly upregulated in B-cell non-Hodgkin lymphomas and is in direct relation to factor HIF1alpha and HIF2alpha expression. LDH5 expression is linked with activated VEGFR2/KDR expression in both lymphoid lesions.

18821170 The expression of LDH and its isoenzymes in pleural effusions reflects the host reaction in pleural space and, in non-small-cell lung cancer, may also feature the anaerobic phenotype of cancer cells.

18814027 Modulation of LDH expression involves alpha6beta4 integrin-FAK-p38MAPK pathway.

18534967 LDL-M is released into blood fo patients exposed to myocardial ischemia reperfusion.

18521687 The results of the current study show that LDH-5 expression may be a useful

prognostic factor for patients with gastric carcinoma.
17483170 biophysical study of ligand binding and protein dynamics in lactate dehydrogenase
17178662 LDH1 was decreased in essential thrombocythemia. This isoenzymatic pattern could be expression of a metabolic adaptation.
17178662 LDH5 was reduced in idiopathic myelofibrosis. This isoenzymatic pattern could be expression of a metabolic adaptation [LDH5]
16766262 Reduction in LDH-A activity resulted in stimulation of mitochondrial respiration and decrease of mitochondrial membrane potential.The tumorigenicity of the LDH-A-deficient cells was severely diminished.
16132575 Lactate dehydrogenase 5 content in tumor cells is directly related to an up-regulated hypoxia inducible factor pathway and is linked with an aggressive phenotype in colorectal adenocarcinomas.
15240094 These data indicate that LDH-A is induced through a non-genomic pathway of estrogen action.
12712614 The activity of this enzyme was studied in tissues, erythrocytes, and blood plasma of patients with peptic ulcer both in its uncomplicated course and in the development of complications.
12629811 The study of this protein in a sportsman is significant for assessment of training efficiency.
12555229 an LDHA exon5 haplotype confers increased risk for paradoxically decreased minute volume respiratory response to CO2 challenge but not to panic disorder
Pathway: Pyruvate metabolism - Homo sapiens (human) (database: KEGG)
Pathway: Glycolysis / Gluconeogenesis - Homo sapiens (human) (database: KEGG)
Pathway: Propanoate metabolism - Homo sapiens (human) (database: KEGG)
Pathway: HIF-1 signaling pathway - Homo sapiens (human) (database: KEGG)
Pathway: Cysteine and methionine metabolism - Homo sapiens (human) (database: KEGG)
Pathway: Cori Cycle (database: Wikipathways)
Pathway: Glycolysis and Gluconeogenesis (database: Wikipathways)
Pathway: hypoxia-inducible factor in the cardivascular system (database: BioCarta)
Pathway: TCR (database: NetPath)
Pathway: Pyruvate metabolism (database: Reactome)
Pathway: Validated targets of C-MYC transcriptional activation (database: PID)
Pathway: Pyruvate metabolism and Citric Acid (TCA) cycle (database: Reactome)
Pathway: The citric acid (TCA) cycle and respiratory electron transport (database: Reactome)
Pathway: Methionine Cysteine metabolism (database: INOH)
Pathway: Propanoate metabolism (database: INOH)
Pathway: Glycolysis Gluconeogenesis (database: INOH)
Pathway: EGFR1 (database: NetPath)
Pathway: Pyruvate metabolism (database: INOH)
Pathway: pyruvate fermentation to lactate (database: HumanCyc)
Pathway: HIF-1-alpha transcription factor network (database: PID)
Pathway: hypoxia-inducible factor in the cardivascular system (database: PID)
Protein: **LDHA** (P00338) L-lactate dehydrogenase A chain;
EC: **1.1.1.27** L-lactate dehydrogenase. (S)-lactate + NAD(+) = pyruvate + NADH.
GO: 0004459 L-lactate dehydrogenase activity
GO: 0005515 protein binding
GO: 0005634 nucleus
GO: 0005739 mitochondrion
GO: 0005829 cytosol
GO: 0005929 cilium
GO: 0006090 pyruvate metabolic process
GO: 0006096 glycolytic process
GO: 0021762 substantia nigra development
GO: 0031668 cellular response to extracellular stimulus
GO: 0044237 cellular metabolic process
GO: 0044281 small molecule metabolic process
GO: 0070062 extracellular vesicular exosome
KO: **K00016** L-lactate dehydrogenase [EC:1.1.1.27]
ReactionKEGG: **R00703** (S)-Lactate + NAD+ <=> Pyruvate + NADH + H+
ReactionKEGG: **R01000** 2-Hydroxybutanoic acid + NAD+ <=> 2-Oxobutanoate + NADH + H+
ReactionKEGG: **R03104** 3-Mercaptolactate + NAD+ <=> Mercaptopyruvate + NADH + H+
OMIM: **150000** LACTATE DEHYDROGENASE A; LDHA
OMIM: **612933** GLYCOGEN STORAGE DISEASE XI; GSD11

footnotes:
- Linkage disequilibrium R2 is based on founders in HapMap r27 CEU population