Identification of driver genes and biological signaling for alcoholic myopathy

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Abstract

Long-term alcohol consumption contributes to muscle weakness and atrophy. However, the mechanism and biological functions are still not clear. In this study, we aim to identify the significantly changed genes and potential signaling pathways in the gastrocnemius and plantaris muscle from C57BL/6Hsd mice by analyzing RNA sequence. The GSE183665 dataset was created by using the Illumina NovaSeq 6000 (*Mus musculus*). The KEGG and GO analyses showed that "cell migration", "cell adhesion", and "apoptosis" are major biological processes in the skeletal muscles. Moreover, we identified a number of genes including POSTN, GNAI2, MMP2, ELN, CCND1, CXCL12, COL6A1, COL6A2, SFRP2, and FSTL1 by using the PPI network and Reactome map. Thus, our study may shed light on the development of drugs on alcohol myopathy.

Introduction

Alcohol regulates the biological function of numerous organs and tissues in the body by interaction with cellular components and relative signaling pathways including oxidative and inflammatory signaling¹. The metabolism of alcohol contributes to the production of acetaldehyde and reactive oxygen that damage organs and tissues such as muscles². The oxidative stress originates from various tissues and organs, which depends on the inflammatory and oxidative state and immune levels³. Additionally, the degree and duration of stress are dependent on the metabolic state of tissues to clear alcohol and its byproducts⁴. Other oxidative stress originates from the increased release of cytokines by the immune cells responding to alcohol⁵. Alcohol is related to signal transduction through cell membranes, signaling receptors, and ion channels⁶. Modification of these receptors and signaling molecules results in changing multiple signaling pathways.

Alcoholism is a major reason for muscle myopathy that is categorized as a decreased skeletal muscle protein synthesis and myofibrillary protein contents⁷. The major affected muscles are rich in type II muscle fibers including plantaris and gastrocnemius⁸. The muscular weakness caused by alcohol may be due to the downregulation of contractile processes. Moreover, the therapeutic strategies targeting muscles may lead to a deleterious effect on others⁹. Therefore, gaining knowledge on the responses to alcohol in muscles may benefit the clinical work.

In our study, we evaluated the effects of alcohol stress on the muscle tissues by analyzing the RNA-seq data. We identified a number of DEGs and significant signaling pathways. We also performed the gene function enrichment and constructed the protein-protein interaction (PPI) network and Reactome map to find the valuable drug targets and pathways. The functional genes and biological processes will guide the clinical work on the treatment of muscle damage caused by alcohol.

Methods

Data resources

The data was obtained by using the Illumina NovaSeq 6000 (Mus musculus) (Biomedical Sciences, Florida State University, 1115 W. Call Street, Tallahassee, Florida, US). The analyzed dataset includes five control groups and five alcohol-treated groups.

Data acquisition and preprocessing

The raw data were processed by the R package as described¹⁰. A classical t-test was performed to identify DEGs with $P < 0.01$ and fold change ≥ 1 as being statistically significant.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses

The KEGG and GO analyses were performed by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (<http://david.ncifcrf.gov/>). We set the $P < .05$ and gene counts > 10 as the statistically significant cutoff.

Protein-protein interaction (PPI) analysis

The PPI was constructed by using the Molecular Complex Detection (MCODE). The biological processes analysis was performed by Reactome (<https://reactome.org/>), and $P < 0.05$ was considered as the statistically significant cutoff.

Results

Identification of DEGs in control and alcohol-treated muscles from C57BL/6Hsd mice

To determine the molecular mechanism of alcohol on muscles, we analyzed the DEGs from the control and alcohol-treated muscles from mice. A total of 360 genes were identified with the threshold of $P < 0.01$. The top ten of up-and-down-regulated genes for control and alcohol-treated muscles are shown by the heatmap and volcano plot (Figure 1). The top ten DEGs were listed in Table 1.

Enrichment analysis of DEGs in control and alcohol treated muscles from C57BL/6Hsd mice

To determine the biological functions of DEGs in muscles with the alcohol treatment, we performed the KEGG and GO analyses (Figure 2). The significant KEGG signaling contained "Herpes simplex virus 1 infection" and "PI3K-Akt signaling pathway". We further determined the significant biological processes of GO including "Ameboidal-type cell migration", "Extracellular matrix organization", "Extracellular structure organization", "External encapsulating structure organization", "Regulation of apoptotic signaling pathway", "Cell-substrate adhesion", "Peptidyl-tyrosine modification", "Peptidyl-tyrosine phosphorylation", "Regulation of peptidyl-tyrosine phosphorylation", and "Regulation of extrinsic apoptotic signaling pathway". We then determined the significant cellular components of GO including "Collagen-containing extracellular matrix", "Neuron to neuron synapse", "Asymmetric synapse",

“Membrane raft”, “Membrane microdomain”, “Postsynaptic density”, “Basement membrane”, “Collagen trimer”, and “Microfibril”. We finally determined the significant molecular functions of GO including “Transcription coregulator activity”, “DNA-binding transcription repressor activity”, “DNA-binding transcription repressor activity, RNA polymerase II-specific”, “Glycosaminoglycan binding”, “Sulfur compound binding”, “Extracellular matrix structural constituent”, “Heparin binding”, “Integrin binding”, “Growth factor binding”, and “Extracellular matrix structural constituent conferring tensile strength”.

PPI network analysis

To further understand the relationship among the DEGs, we constructed the PPI network by using the 297 nodes and 206 edges (String network). The criterion of combined score > 0.4 was defined to create the PPI in the Cytoscape software. Table 2 indicated the top ten genes with the highest degree scores. The top two modules showed the functional annotation (Figure 3). We further analyzed the DEGs and PPI networks by using the Reactome map (Figure 4). We identified the top ten significant biological processes including “Extra-nuclear estrogen signaling”, “ESR-mediated signaling”, “Activation of Matrix Metalloproteinases”, “NOTCH3 Intracellular Domain Regulates Transcription”, “Activation of SMO”, “Molecules associated with elastic fibers”, “Signal amplification”, “Signaling by NOTCH3”, “RUNX3 regulates WNT signaling”, and “RET signaling” (Supplemental Table S1).

Discussion

Alcoholism leads to a kind of myopathic lesion characterized by selective atrophy of Type II fibers¹¹. Moreover, alcoholism causes reductions in muscle mass and body mass¹². Muscle strength may also be impaired by alcohol with long-term intake¹³. Our study mainly focused on the mechanism and impact of alcoholism on the muscles, which may help to develop drugs by targeting myopathy.

In this study, we found alcohol mainly affects cell migration, cell adhesion, and apoptosis by analyzing the KEGG and GO enrichment. Similarly, Tatsuro Kumada et al found that alcohol affects the migration of immature neurons¹⁴. Moreover, the abnormal migration of neurons by alcohol can be improved by regulating the second-messenger pathways¹⁴. R Ramanathan et al found that alcohol can completely repress the L1-mediated cell-cell adhesion¹⁵. Ana Rodriguez et al found that the high alcohol level leads to increased caspase-3 activity and declined contractility, whereas the low alcohol level is related to the decreased apoptosis¹⁶. It is suggested that the low alcohol level may have the potential to protect the muscles by regulating the cell functions.

By analyzing the PPI network, we also identified several potential DEGs that may affect the function of muscles by alcohol intake. The study by Masamitsu Hara et al showed that the periostin enhances the migration of fibroblast and represses the repair after injury¹⁷. Another finding showed the periostin is expressed in the cardiac fibroblasts, which can result in cardiomyocyte maturation and innervation¹⁸. G-

protein-coupled receptors (GPCRs), Regulators of G protein signaling (RGS), and their downstream pathways are involved in various physiological and pathological processes^{19–21} including inflammation, metabolism, cancer, and pain^{22–28}. As a significant heterotrimeric G protein, GNAI2 ameliorates cell proliferation and inhibits apoptosis processes²⁶. Elin Hadler-Olsen et al found MMP2 is related to the type II fibers through regulating the extracellular substrates²⁹. MMP2 plays an important role in skeletal muscle cell migration and differentiation³⁰. Circadian gene clocks and their downstream genes are associated with a number of signaling pathways such as metabolism, immune, and aging processes^{31–40}. Interestingly, as a circadian clock-controlled gene, MMP2 shows aberrant functions in clock mutant mice⁴¹. Elastin is expressed in G0 and G2/M phases of vascular smooth muscle cells, which can further regulate the cell proliferation ability⁴². Jamie O Brett et al found that Cyclin D1 maintains the muscle stem cell activation by inhibiting TGF signaling⁴³. The study by Kyuho Jeong et al found the nuclear FAK can regulate the vascular smooth muscle cell proliferation and hyperplasia by mediating Cyclin D1 levels⁴⁴. Muscle-derived CXCL12 controls the proliferation of endothelial cells but not the angiogenesis⁴⁵. CXCL12 and its receptor CXCR4 are critical for the recruitment and development of progenitor stem cells of skeletal muscles and bones²³. NF- κ B is an essential molecule that regulates various immune-related diseases such as rheumatoid arthritis, osteoarthritis, and periodontitis. Lisa A Madge et al found that the canonical NF- κ B negatively mediates the noncanonical NF- κ B-regulated CXCL12 expression⁴⁶. Kate M D Bushby found that the mutations of COL6A1 and COL6A2 can result in muscle disorders including the ullrich congenital muscular dystrophy and Bethlem myopathy⁴⁷. COL6A1 and COL6A2 are reported to be related to the severity of collagen VI myopathies⁴⁸. J M Levin found that SFRP2 mRNA is highly expressed in the embryonic muscle cells but is lowly expressed in the C2.7 cell line⁴⁹. As a secreted muscle protein, FSTL1 enhances endothelial cell function via a nitric-oxide synthase-dependent mechanism⁵⁰.

In conclusion, this study provided novel knowledge on the impact of muscles by alcoholism. Alcohol mainly affects the cell migration, cell adhesion, and apoptosis processes in the skeletal muscles. Our study may contribute to clinical research on alcoholic myopathy.

Declarations

Author Contributions

Jing Li, Letian Wang: Methodology and Writing. Hanming Gu: Conceptualization, Methodology, Writing-Reviewing and Editing.

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Declarations of interest

There is no conflict of interest to declare.

References

1. Szabo G, Saha B: Alcohol's Effect on Host Defense. *Alcohol Res* 2015, 37:159–70.
2. Zakhari S: Overview: how is alcohol metabolized by the body? *Alcohol Res Health* 2006, 29:245–54.
3. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A: Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev* 2017, 2017:8416763.
4. Koop DR: Alcohol metabolism's damaging effects on the cell: a focus on reactive oxygen generation by the enzyme cytochrome P450 2E1. *Alcohol Res Health* 2006, 29:274–80.
5. Crews FT, Lawrimore CJ, Walter TJ, Coleman LG, Jr.: The role of neuroimmune signaling in alcoholism. *Neuropharmacology* 2017, 122:56–73.
6. Ron D, Messing RO: Signaling pathways mediating alcohol effects. *Curr Top Behav Neurosci* 2013, 13:87–126.
7. Steiner JL, Lang CH: Dysregulation of skeletal muscle protein metabolism by alcohol. *Am J Physiol Endocrinol Metab* 2015, 308:E699-712.
8. Wang Y, Pessin JE: Mechanisms for fiber-type specificity of skeletal muscle atrophy. *Curr Opin Clin Nutr Metab Care* 2013, 16:243–50.
9. Min YL, Bassel-Duby R, Olson EN: CRISPR Correction of Duchenne Muscular Dystrophy. *Annu Rev Med* 2019, 70:239–55.
10. Hanming G: neutrophils arthritis. *Research Square* 2021.
11. Preedy VR, Adachi J, Ueno Y, Ahmed S, Mantle D, Mullatti N, Rajendram R, Peters TJ: Alcoholic skeletal muscle myopathy: definitions, features, contribution of neuropathy, impact and diagnosis. *Eur J Neurol* 2001, 8:677–87.
12. Simon L, Jolley SE, Molina PE: Alcoholic Myopathy: Pathophysiologic Mechanisms and Clinical Implications. *Alcohol Res* 2017, 38:207–17.
13. Buchmann N, Spira D, König M, Norman K, Demuth I, Steinhagen-Thiessen E: Problematic drinking in the old and its association with muscle mass and muscle function in type II diabetes. *Sci Rep* 2019, 9:12005.
14. Kumada T, Jiang Y, Cameron DB, Komuro H: How does alcohol impair neuronal migration? *J Neurosci Res* 2007, 85:465–70.
15. Ramanathan R, Wilkemeyer MF, Mittal B, Perides G, Charness ME: Alcohol inhibits cell-cell adhesion mediated by human L1. *J Cell Biol* 1996, 133:381–90.

16. Rodriguez A, Chawla K, Umoh NA, Cousins VM, Ketegou A, Reddy MG, AlRubaiee M, Haddad GE, Burke MW: Alcohol and Apoptosis: Friends or Foes? *Biomolecules* 2015, 5:3193-203.
17. Hara M, Yokota K, Saito T, Kobayakawa K, Kijima K, Yoshizaki S, Okazaki K, Yoshida S, Matsumoto Y, Harimaya K, Nakashima Y, Okada S: Periostin Promotes Fibroblast Migration and Inhibits Muscle Repair After Skeletal Muscle Injury. *J Bone Joint Surg Am* 2018, 100:e108.
18. Hortells L, Valiente-Alandi I, Thomas ZM, Agnew EJ, Schnell DJ, York AJ, Vagnozzi RJ, Meyer EC, Molkentin JD, Yutzey KE: A specialized population of Periostin-expressing cardiac fibroblasts contributes to postnatal cardiomyocyte maturation and innervation. *Proc Natl Acad Sci U S A* 2020, 117:21469–79.
19. Yuan G, Yang S, Yang S: Macrophage RGS12 contributes to osteoarthritis pathogenesis through enhancing the ubiquitination. *Genes & Diseases* 2021.
20. Yuan G, Yang S, Ng A, Fu C, Oursler MJ, Xing L, Yang S: RGS12 Is a Novel Critical NF-kappaB Activator in Inflammatory Arthritis. *iScience* 2020, 23:101172.
21. Fu C, Yuan G, Yang ST, Zhang D, Yang S: RGS12 Represses Oral Cancer via the Phosphorylation and SUMOylation of PTEN. *J Dent Res* 2020:22034520972095.
22. Fan XF, Wang XR, Yuan GS, Wu DH, Hu LG, Xue F, Gong YS: [Effect of safflower injection on endoplasmic reticulum stress-induced apoptosts in rats with hypoxic pulmonary hypertension]. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 2012, 28:561–7.
23. Gilbert W, Bragg R, Elmansi AM, McGee-Lawrence ME, Isales CM, Hamrick MW, Hill WD, Fulzele S: Stromal cell-derived factor-1 (CXCL12) and its role in bone and muscle biology. *Cytokine* 2019, 123:154783.
24. Yuan G, Yang S, Yang S, Ng A, Oursler MJ: RGS12 is a critical proinflammatory factor in the pathogenesis of inflammatory arthritis via acting in Cox2-RGS12-NF kappa B pathway activation loop. *J Bone Miner Res: WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA*, 2019. pp. 147-.
25. Yuan G, Fu C, Yang ST, Yuh DY, Hajishengallis G, Yang S: RGS12 Drives Macrophage Activation and Osteoclastogenesis in Periodontitis. *J Dent Res* 2021:220345211045303.
26. Hu S, Dai Y, Bai S, Zhao B, Wu X, Chen Y: GNAI2 Promotes Proliferation and Decreases Apoptosis in Rabbit Melanocytes. *Genes (Basel)* 2021, 12.
27. Yuan G, Yang S, Liu M, Yang S: RGS12 is required for the maintenance of mitochondrial function during skeletal development. *Cell Discov* 2020, 6:59.
28. Yuan G, Yang S, Gautam M, Luo W, Yang S: Macrophage regulator of G-protein signaling 12 contributes to inflammatory pain hypersensitivity. *Ann Transl Med* 2021, 9:448.
29. Hadler-Olsen E, Solli AI, Hafstad A, Winberg JO, Uhlin-Hansen L: Intracellular MMP-2 activity in skeletal muscle is associated with type II fibers. *J Cell Physiol* 2015, 230:160–9.
30. Chen X, Li Y: Role of matrix metalloproteinases in skeletal muscle: migration, differentiation, regeneration and fibrosis. *Cell Adh Migr* 2009, 3:337–41.

31. Zhu Z, Hua B, Xu L, Yuan G, Li E, Li X, Sun N, Yan Z, Lu C, Qian R: CLOCK promotes 3T3-L1 cell proliferation via Wnt signaling. *IUBMB Life* 2016, 68:557–68.
32. Yuan G, Hua B, Yang Y, Xu L, Cai T, Sun N, Yan Z, Lu C, Qian R: The Circadian Gene Clock Regulates Bone Formation Via PDIA3. *J Bone Miner Res* 2017, 32:861–71.
33. Xu L, Cheng Q, Hua B, Cai T, Lin J, Yuan G, Yan Z, Li X, Sun N, Lu C, Qian R: Circadian gene Clock regulates mitochondrial morphology and functions by posttranscriptional way. *bioRxiv* 2018:365452.
34. Mao SZ, Fan XF, Xue F, Chen R, Chen XY, Yuan GS, Hu LG, Liu SF, Gong YS: Intermedin modulates hypoxic pulmonary vascular remodeling by inhibiting pulmonary artery smooth muscle cell proliferation. *Pulm Pharmacol Ther* 2014, 27:1–9.
35. Yuan G, Hua B, Cai T, Xu L, Li E, Huang Y, Sun N, Yan Z, Lu C, Qian R: Clock mediates liver senescence by controlling ER stress. *Aging* 2017, 9:2647–65.
36. Zhu Z, Hua B, Shang Z, Yuan G, Xu L, Li E, Li X, Sun N, Yan Z, Qian R, Lu C: Altered Clock and Lipid Metabolism-Related Genes in Atherosclerotic Mice Kept with Abnormal Lighting Condition. *Biomed Res Int* 2016, 2016:5438589.
37. Yuan G, Xu L, Cai T, Hua B, Sun N, Yan Z, Lu C, Qian R: Clock mutant promotes osteoarthritis by inhibiting the acetylation of NFkappaB. *Osteoarthritis Cartilage* 2019, 27:922–31.
38. Cai T, Hua B, Luo D, Xu L, Cheng Q, Yuan G, Yan Z, Sun N, Hua L, Lu C: The circadian protein CLOCK regulates cell metabolism via the mitochondrial carrier SLC25A10. *Biochim Biophys Acta Mol Cell Res* 2019, 1866:1310–21.
39. Zhu Z, Xu L, Cai T, Yuan G, Sun N, Lu C, Qian R: Clock represses preadipocytes adipogenesis via GILZ. *J Cell Physiol* 2018, 233:6028–40.
40. Yuan G: Identification of biomarkers and pathways of mitochondria in sepsis patients. *bioRxiv* 2021:2021.03.29.437586.
41. Anea CB, Ali MI, Osmond JM, Sullivan JC, Stepp DW, Merloiu AM, Rudic RD: Matrix metalloproteinase 2 and 9 dysfunction underlie vascular stiffness in circadian clock mutant mice. *Arterioscler Thromb Vasc Biol* 2010, 30:2535–43.
42. Tajima S: Modulation of elastin expression and cell proliferation in vascular smooth muscle cells in vitro. *Keio J Med* 1996, 45:58–62.
43. Brett JO, Arjona M, Ikeda M, Quarta M, de Morree A, Egner IM, Perandini LA, Ishak HD, Goshayeshi A, Benjamin DI, Both P, Rodriguez-Mateo C, Betley MJ, Wyss-Coray T, Rando TA: Exercise rejuvenates quiescent skeletal muscle stem cells in old mice through restoration of Cyclin D1. *Nat Metab* 2020, 2:307–17.
44. Jeong K, Kim JH, Murphy JM, Park H, Kim SJ, Rodriguez YAR, Kong H, Choi C, Guan JL, Taylor JM, Lincoln TM, Gerthoffer WT, Kim JS, Ahn EE, Schlaepfer DD, Lim SS: Nuclear Focal Adhesion Kinase Controls Vascular Smooth Muscle Cell Proliferation and Neointimal Hyperplasia Through GATA4-Mediated Cyclin D1 Transcription. *Circ Res* 2019, 125:152–66.
45. Yamada M, Hokazono C, Tokizawa K, Marui S, Iwata M, Lira VA, Suzuki K, Miura S, Nagashima K, Okutsu M: Muscle-derived SDF-1alpha/CXCL12 modulates endothelial cell proliferation but not

- exercise training-induced angiogenesis. *Am J Physiol Regul Integr Comp Physiol* 2019, 317:R770-R9.
46. Madge LA, May MJ: Classical NF-kappaB activation negatively regulates noncanonical NF-kappaB-dependent CXCL12 expression. *J Biol Chem* 2010, 285:38069–77.
 47. Bushby KM, Collins J, Hicks D: Collagen type VI myopathies. *Adv Exp Med Biol* 2014, 802:185–99.
 48. Butterfield RJ, Foley AR, Dastgir J, Asman S, Dunn DM, Zou Y, Hu Y, Donkervoort S, Flanigan KM, Swoboda KJ, Winder TL, Weiss RB, Bonnemann CG: Position of glycine substitutions in the triple helix of COL6A1, COL6A2, and COL6A3 is correlated with severity and mode of inheritance in collagen VI myopathies. *Hum Mutat* 2013, 34:1558–67.
 49. Levin JM, El Andalousi RA, Dainat J, Reyne Y, Bacou F: SFRP2 expression in rabbit myogenic progenitor cells and in adult skeletal muscles. *J Muscle Res Cell Motil* 2001, 22:361–9.
 50. Ouchi N, Oshima Y, Ohashi K, Higuchi A, Ikegami C, Izumiya Y, Walsh K: Follistatin-like 1, a secreted muscle protein, promotes endothelial cell function and revascularization in ischemic tissue through a nitric-oxide synthase-dependent mechanism. *J Biol Chem* 2008, 283:32802–11.

Tables

Tables 1-2 are in the supplementary files section.

Figures

Figure 1

Identification of DEGs in control and alcohol treated muscles from C57BL/6Hsd mice (A) Heatmap of DEGs between control and alcohol treated muscles from C57BL/6Hsd mice. Regularized matrix was generated using the R package. (B) Volcano plot for DEGs between control and alcohol treated muscles from C57BL/6Hsd mice. The most significant genes are marked with symbols.

Figure 2

Gene enrichment analysis in control and alcohol-treated muscles from C57BL/6Hsd mice (A) KEGG analysis was performed by the R package. (B) Different colors represent biological processes (BP). (C) The cellular components (CC) were analyzed by the R package. (D) The molecular functions (MF) were analyzed by the R package.

Figure 3

The PPI network analysis between control and alcohol-treated muscles from C57BL/6Hsd mice 297 nodes and 206 edges were obtained from the STRING database for creating the PPI network. Cluster 1 (A) and cluster 2 (B) were constructed by Cytoscape.

Figure 4

Reactome map indication of the significant biological processes of the protein elements identified between control and alcohol-treated muscles from C57BL/6Hsd mice

Supplementary Files

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