Rat mitochondrial ATP synthase ATP5G3: cloning and upregulation in pancreas after chronic ethanol feeding

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EXCESSIVE INTAKE OF ALCOHOL often precedes the development of both acute and chronic pancreatitis, and pancreatitis occurs more commonly in alcoholics than in the general population (17, 37). Thus alcohol appears to be one of the major risk and etiological factors in the pathogenesis of human pancreatitis (30, 31). However, little is known about molecular mechanisms whereby alcohol alters pancreatic cell biology and therefore predisposes consumers to pancreatic injury and/or pancreatitis. Furthermore, chronic pancreatitis can only be diagnosed when the disease is fully established, so that the underlying pathology is relatively unknown.

METHODS

The mitochondrion is one of the most vulnerable intracellular targets to alcohol-induced injury. Such injury could lead to potential energy deficits and oxidative stress (5, 12, 25), which are of special concern in the pancreas because of the high energy demand for protein synthesis. Indeed, giant mitochondria have been observed in pancreatic exocrine cells in rats receiving both ethanol and carbonyl iron (41), suggesting toxin-induced mitochondrial damage. It has been suggested that abnormal mitochondrial structure may contribute to the dysfunction of the pancreatic acinar cells in alcoholism (41). However, reports on morphological and functional changes of pancreatic mitochondria caused by ethanol intake are inconsistent (39, 47). Furthermore, the key molecular components in mitochondria associated with the alcohol-induced cellular damage in the pancreas are unknown.

The purpose of this study was to identify and isolate key molecules associated with cellular responses to chronic alcohol consumption in the pancreas of rats and thereby to gain insight into the possible pathogenesis of human pancreatitis. Alcohol-induced differential gene expression in the pancreas may also suggest an important adaptive process in the compensatory pancreatic function in response to chronic alcohol ingestion and thus provide clues to possible protective mechanisms that prevent the development of alcohol-related pancreatitis in most human alcoholics. Through gene expression profiling using mRNA differential display, we have identified and cloned a rat gene that was overexpressed in the pancreas of rats chronically fed ethanol for 4 wk. The cDNA sequence encodes a novel rat mitochondrial ATP synthase subunit 9, isoform 3 gene (ATP5G3), a nuclear gene encoding a mitochondrial protein. Differential expression of this gene suggests a cellular response to mitochondrial damage and metabolic stress in the pancreas of alcohol-consuming rats and indicates a possible adaptive mechanism for mitochondrial repair.

Animal model and tissue collection. Male Wistar rats weighing 80–110 g (Harlan Sprague-Dawley, Indianapolis, IN) were individually housed in a temperature-controlled (23 ± 2°C) and 12:12-h light/dark room. The rats were pair-
fed with nutritionally adequate liquid diets (Lieber-DeCarli diet) (26) (Bio-Serv, Frenchtown, NJ). Alcohol-fed rats received 6.7% (vol/vol) ethanol (36% of total calories) in liquid diet for 4 wk. At the end of the feeding period, rats were fasted overnight and the next day given 20 ml of control diet 2 h before they were killed, to enhance differential gene expression in the pancreas after meal stimulation. Tissues from pancreas were dissected rapidly, snap-frozen in liquid nitrogen, and stored at −80°C or fixed for electron microscopy. Animal maintenance and experiments were performed following protocols approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh, Pittsburgh, PA.

Transmission electron microscopy. Pancreatic pellets were cut into 1-mm³ cubes and immersion-fixed in 2.5% glutaraldehyde for several days at 4°C. Tissue was then washed three times in PBS, postfixed in aqueous 1% OsO₄ and 1% K₃Fe(CN)₆ for 1 h. Following three PBS washes, the pellet was dehydrated through a graded series of 30–100% ethanol, 100% propylene oxide, then infiltrated in a 1:1 mixture of propylene oxide:Polybed 812 epoxy resin (Polysciences, War- rington, PA) for 1 h. After several changes of 100% resin over 24 h, the pellet was embedded in a mold, cured at 37°C overnight, followed by additional hardening at 65°C for 2 days. Ultrathin (60 nm) sections of tissue (500 μm × 500 μm) were collected on 200-mesh copper grids, stained with 2% uranyl acetate in 50% methanol for 10 min, and followed by 1% lead citrate for 7 min. Sections were analyzed and photographed using a transmission electron microscope (model JEM 1210; JEOL, Peabody, MA) at 80 or 60 kV onto electron microscope film (ESTAR thick base; Kodak, Rochester, NY).

mRNA differential display. Technical details about mRNA differential display were described in a recent publication (24). Briefly, tissue was individually homogenized in TRIzol reagent (GIBCO-BRL, Gaithersburg, MD) using pestles and microcentrifuge tubes (roughened in our laboratory). Total RNA was extracted using TRIzol reagent following the instructions of the manufacturer. Integrity of total RNA was examined in 1% TBE agarose gel (TBE is 1× Tris base, 0.02 M EDTA, disodium, 1 M boric acid, pH 8.3) (Biofluids, Rock- ville, MD). Reagents for mRNA differential display were supplied in RNAImage kits (GenHunter, Nashville, TN). The RNA was treated with RNase-free DNase I to reduce the risk for genomic DNA contamination. The RT reaction included 2 μg RNA-free total RNA, a differential display oligo(dT) primer and an arbitrary primer, α-32P-labeled dATP (1,200 Ci/mmole) (Du Pont NEN, Boston, MA) or [α-32P]dATP (3,000 Ci/mmol, ICN Biomedicals, Costa Mesa, CA), and AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT). Low-stringency PCR was performed for 40 cycles with temperature at 94°C for 15 s, at 40°C for 2 min, and at 72°C for 2 min on a thermocycler (model PE 480; Perkin-Elmer Applied Biosystems, Foster City, CA). The PCR reaction included 2 μl of cDNA, a differential display oligo(dT) primer and an arbitrary primer, α-32P-labeled dATP (1,200 Ci/mmole) (Du Pont NEN, Boston, MA) or [α-32P]dATP (3,000 Ci/mmol) (ICN Biomedicals, Costa Mesa, CA), and AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT). Low-stringency PCR was performed for 40 cycles with temperature at 94°C for 15 s, at 40°C for 2 min, and at 72°C for 2 min on a thermocycler (model PE 9600). PCR products were size-fragmented on a 6% denaturing polyacrylamide gel (National Diagnostics, Atlanta, GA) for 3.5 h. The gel was dried under vacuum at 80°C and exposed to a Kodak XAR-2 film (Scientific Imaging Systems, Rochester, NY) for 24–48 h. The autoradiogram with bands showing differential expression was aligned with the gel with the aid of Glogos II Autorad Markers (Stratagene, La Jolla, CA). Differentially expressed bands were excised from the gel, and the cDNA was extracted by ethanol precipitation and centrifugation. The cDNA was reamplified by PCR as previously described (24).

Cloning and sequencing. A PCR product containing a cDNA of interest was purified using the Wizard PCR Prep DNA Purification System (Promega, Madison, WI). The purified PCR product was cloned using pGEM-T Vector System II (Promega). Positive colony selection, plasmid DNA preparation, and sequencing were performed as previously described (24).

Construction and screening of a rat pancreatic cDNA library. Total RNA was extracted from the pancreas of control and ethanol-fed rats. The poly(A⁺) RNA was purified, and a directional cDNA library was constructed in the EcoRI/XhoI sites of Uni-ZAP XR vectors (Stratagene). The titer of the amplified cDNA library was 1.9 × 10⁹ pfu/ml, and the average insert size was 1 kb. Phage DNA was purified following instructions (35) and used as a template for PCR screening. One cDNA that was upregulated in the pancreas of ethanol-fed rats was isolated, cloned, and sequenced. The clone sequence was highly homologous at 3' noncoding region to a human mitochondrial ATP synthase F₆ complex subunit 9, isofrom 3 (ATP5G3), mRNA (GenBank accession no. U99813) (50). Because there was no rat ATP5G3 cDNA sequence available in GenBank databases, for ATP5G3 coding sequence information, the rat pancreatic cDNA library was screened via PCR with ATP5G3 gene-specific primers (Table 1) that were designed based on the human ATP5G3 cDNA sequence (U99813) (50). The amplified PCR products were subcloned using pGEM-T Vector System II (Promega) and sequenced.

Data analysis. Analyses of cDNA sequences, functional motifs, and restriction mapping were performed using Sequencer 3.1 software (Gene Codes, Ann Arbor, MI), BLAST (http://www.ncbi.nlm.nih.gov/blast) (2), and MOTIF (http://www.motif.genome.ad.jp).

RNA slot blot. Total RNA (1.0 μg) was extracted from the pancreas of two control and two ethanol-fed rats. The RNA was applied onto a positively charged nylon membrane (Ambion, Austin, TX) using a Bio-Dot microfiltration system (Bio-Rad, Hercules, CA). The membrane was dried at 80°C under vacuum and cross-linked by ultraviolet light (254 nm, 120 mJ/cm²) in a cross linker (Stratagene). A cDNA probe (401 bp) was generated by PCR amplification using ATP5G3 gene-specific primers (Primer set 4, Table 1) from the rat pancreatic cDNA library. The cDNA was subcloned and sequenced. The plasmid DNA was labeled with [α-32P]dCTP (3,000 Ci/mmol, ICN) during PCR and purified using a Quick Spin Column, Sephadex G-50 (Fine) (Boehringer Mannheim, Indianapolis, IN) to remove unincorporated nucleotides. Duplicate membranes containing the total RNA were hybridized to a [α-32P]-labeled probe (specific activity 10⁶ cpm/ml). Hybridization was performed in a rapid-Hyb buffer (Amersham Life Science, Arlington Heights, IL) for 2.5 h at 42°C. The membrane was washed twice for 30 min at room temperature with a buffer containing 2× SSC/0.1% (wt/vol) SDS and two times for 30 min at 65°C with a buffer containing 0.5× SSC/0.1% SDS. The membranes were then assembled and exposed to a Kodak XAR-2 film (Scientific Imaging Systems) with an intensifying screen at −80°C. Signal intensity from the autoradiogram was determined by image scanning (Agfa NDT, Ridgefield Park, NJ), and hybridization patterns were compared.

Real-time PCR. Because ATP synthase subunit 9 was encoded by three transcripts (ATP5G1, ATP5G2, and ATP5G3), gene-specific primers were designed at the regions of leader sequences using Primer Express (Version 1.0, PE Applied Biosystems) (Table 2) to determine the relative ex-
pression levels of each gene family member in the pancreas in this model. As a standard internal control, primers for a rat 18S rRNA (GenBank accession no. M11188) (44) were designed, including upstream primer (5'-CCA TTC GAA CGT CTG CCC TAT-3') and downstream primer (5'-GTC ACC CGT GGT CAC CAT G-3'). Total RNA was extracted from the pancreas of two individual rats in each group (control and ethanol fed). Real-time PCR was carried out following a method previously described (24). Briefly, the RT reaction included DNA-free RNA (final concentration at 5 ng/μl), random primer, and SuperScript II reverse transcriptase (GIBCO-BRL). The reaction was incubated at 25°C for 10 min, at 48°C for 30 min, and at 95°C for 5 min on the thermocycler (PE 9600). SYBR Green PCR reagents (PE Applied Biosystems) were used for real-time PCR. Triplicate reactions from each cDNA (5 μl) were prepared for real-time PCR following conditions at 50°C for 2 min, at 95°C for 12 min, and for 40 cycles at 95°C for 15 s and at 60°C for 1 min on an ABI Prism 7700 Sequence Detection system (PE Applied Biosystems). The ABI Prism 7700 Sequence Detection software (PE Applied Biosystems) was used for instrument control, automated data collection, and analysis.

RESULTS

Ultrastructural changes in mitochondria. To investigate the correlation between differential gene expression and subcellular pathological alterations in the pancreas in this model, transmission electron microscopy was performed. Striking changes in the mitochondria were observed in the pancreatic exocrine cells of rats fed ethanol diet for 4 wk, including swelling of mitochondria, fragmented inner membrane, and damaged cristae (Fig. 1).

Isolation of a rat cDNA tag encoding a novel rat mitochondrial ATP5G3 gene. Integrity of pancreatic total RNA was demonstrated in Fig. 2. Routinely checking RNA integrity by electrophoresis is a critical step in our laboratory before performing any downstream experiments. Using mRNA differential display, we isolated one cDNA tag (178 bp) that was overexpressed in the pancreas of rats fed ethanol for 4 wk (Fig. 3). The rat clone cDNA sequence shared 90%

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Table 1. Primers designed based on the human ATP 5G3 cDNA sequence (U09813) (Ref. 50) and used for cloning a rat ATP5G3 cDNA via PCR screening of a rat pancreatic cDNA library

<table>
<thead>
<tr>
<th>Primer set 1</th>
<th>Primer set 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F41, R158</td>
<td>F88, R185</td>
</tr>
<tr>
<td>5'-CCA GTA GGG ACC CAT TCA TTG TGC-3'</td>
<td>5'-CCA AGG TGA CCG GGT GC-3'</td>
</tr>
<tr>
<td>5'-CTG CCG AGG AAA AGA GGA TTA AGG-3'</td>
<td>5'-GCC TCC TCT GCC GCT TCC-3'</td>
</tr>
<tr>
<td>141 bp ApaI</td>
<td>115 bp AflII, ApaLI</td>
</tr>
</tbody>
</table>

Table 2. Primers designed for real-time PCR quantification of the rat ATP5G1, ATP5G2, and ATP5G3 cDNAs

<table>
<thead>
<tr>
<th>Primer set 3</th>
<th>Primer set 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>F116, R560</td>
<td>F235, R612</td>
</tr>
<tr>
<td>5'-CGG AGG CTG GGT GGG AAG A-3'</td>
<td>5'-CAT AGG TCC AGA CAA GGC AAA TCC-3'</td>
</tr>
<tr>
<td>468 bp BamHI</td>
<td>401 bp BamHI</td>
</tr>
</tbody>
</table>

Table 3. Primers designed for real-time PCR quantification of the rat ATP5G1, ATP5G2, and ATP5G3 cDNAs

<table>
<thead>
<tr>
<th>Primer set 5</th>
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</thead>
<tbody>
<tr>
<td>F569, R740</td>
</tr>
<tr>
<td>5'-AAA CCC TTC GCT GGA A-3'</td>
</tr>
<tr>
<td>189 bp Eco31I</td>
</tr>
</tbody>
</table>

Fig. 1. Transmission electron micrographs of mitochondria (arrows). A: typical normal mitochondria in a pancreatic exocrine cell of a pair-fed control rat. B: mitochondria in a pancreatic exocrine cell of a rat fed ethanol diet for 4 wk. Note the increased size of mitochondria, damaged cristae, and fragmentation of the mitochondrial inner membrane.
Cloning a full-length coding sequence of a rat ATP5G3 gene. A cDNA sequence (723 bp) of a novel rat mitochondrial ATP5G3 gene (GenBank accession no. AP315374) was cloned from the rat pancreatic cDNA library, including a full-length coding sequence (429 bp) that was 100% homologous to the human mitochondrial ATP5G3 cDNA (Fig. 4). Species-specific differences occur immediately after the termination signal. The amino acid sequence and a functional motif (ATP synthase subunit signature) of the subunit 9 were also demonstrated (Fig. 4).

Verification of ATP5G3 mRNA differential expression. To verify that the transcript of the mitochondrial ATP5G3 gene was truly differentially expressed in the pancreas of ethanol-fed rats, a second animal feeding study was undertaken. The overexpression of the rat ATP5G3 gene was confirmed by mRNA differential display (data not shown). The major limitation of Northern analysis is the excessive amount of RNA required (at least 10 μg of total RNA needed), which was not always feasible in our study because the amount of RNA available for all experiments was limited. Therefore, RNA slot blot (using only 1 μg RNA) was performed to confirm that this transcript was indeed upregulated (Fig. 5).

Differential expression of nuclear genes that encode ATP synthase subunit 9 isoforms. Results from real-time PCR demonstrated that all three transcripts (ATP5G1, ATP5G2, and ATP5G3) encoding the rat mitochondrial ATP synthase subunit 9 isoforms 1, 2, and 3 were consistently upregulated by about twofold in the pancreata of rats fed ethanol for 4 wk (Fig. 6).

DISCUSSION

In this study, we cloned a novel rat ATP5G3 cDNA that encodes mitochondrial ATP synthase subunit 9. We also demonstrated that this cDNA as well as other two gene family members, ATP5G1 and ATP5G2, were consistently upregulated at transcriptional levels in the pancreas of rats fed ethanol for 4 wk. The transmission electron micrographs provided compelling evidence of the disrupted mitochondrial function in pancreatic acinar cells in this model.

Mitochondria produce most of the cell’s energy by oxidative phosphorylation, a process that requires the orchestrated actions of five respiratory enzyme complexes located in the mitochondrial inner membrane (36). Electrons are passed along a series of respiratory enzyme complexes, and the energy released by this electron transfer is used to pump protons across the membrane. The resultant electrochemical gradient enables the last enzyme complex (complex V, ATP synthase) to synthesize an energy-carrying molecule, ATP. Attempts have been made to model how proton translocation is coupled to electron transfer in the respiratory enzyme complexes on the basis of kinetic, spectroscopic, and inhibitor data, but verification of these models awaits relevant structural information (21, 45).

The proton-transporting ATP synthase, F0F1-ATPase, consists of a proton pathway, F0 (ion motor, α1β2c12) (22), and a catalytic sector, F1 (ATP motor, α3β3γ1δ1ε1). The F0 contains the proton channels that are linked to the catalytic component (F1) located in the matrix side of the membrane (14, 36). Mitochondrial ATP synthase subunit 9 (also called subunit c, protein 9, or proteolipid), a highly hydrophobic protein, is one of the chains of the nonenzymatic membrane components that reside in the transmembrane portion of the F0 complex. Each ATP synthase F0 complex subunit 9 has 12 copies (36, 38). The coupled rotation of the subunit 9 in F0 and subunit gamma in F1 appears to be essential for energy coupling between proton transport though F0 and ATP hydrolysis or synthesis in F1 (34). Therefore, subunit 9 is a key component for F0 complex function (16), like an energy-driving motor.

Mitochondria are not self-supporting entities, but rely heavily on imported nuclear gene products for proper function (40). The coordination of nuclear and mitochondrial genes plays an important role in regulation of mitochondrial biogenesis, disease, and function (10, 23, 40, 46). A coordinated activation and transcription of the mitochondrial and nuclear genes for the components of the respiratory apparatus, cytochrome-c oxidase subunits and ATP synthase subunit 9, begins with the two-cell stage (42), indicating the importance of the coregulation of mitochondrial and nuclear genes in the maintenance of the normal mitochondrial function. The mammalian mitochondrial ATP synthase subunits are transcriptionally regulated by nuclear genes (3, 4). Three nuclear genes, ATP5G1, ATP5G2, and ATP5G3, encoding the mitochondrial ATP synthase subunit 9, have been cloned in human...
and other animal species (Table 3, this study). The transcripts have been specifically detected in normal human tissues from heart, liver, brain, muscle, and pancreas (20). Three isoforms (P1, P2, and P3) of subunit 9 have specified precursors with different import sequences but identical mature proteins (50). The highly conserved protein sequences occurring across various species, especially the functional motif (ATP synthase c subunit signature, Fig. 4), indicate the vital role of the subunit 9 in the maintenance of the normal mitochondrial physiological function.

Tissue-specific alterations in mitochondrial oxidative phosphorylation and related enzymes and genes have been studied in alcoholic humans and animal models (6, 13, 19). Enzyme activities of the mitochondrial ATP synthase and the mitochondrial DNA were increased in the cardiac tissue, but decreased in the brain and liver, in alcohol-fed animals (28). Nuclear gene products of ATP synthase subunits were unaffected, but mitochondrial-encoded subunits 6 and 8 were depressed, in the livers of ethanol-fed rats (7). It was also found that inhibition of the respiratory activity correlates with the lower activities of the ATP synthase in liver mitochondria of ethanol-fed rats (27).

The effects of chronic ethanol consumption on oxidative phosphorylation in the pancreas may be similar to liver but differ compared with other organs. Pancreas and

![Fig. 4. Sequence homologies of the mitochondrial ATP5G3 cDNAs between rat (AF315374) (this study) and human (U09813) (50). A rat clone sequence (178 bp) isolated by mRNA differential display is demonstrated, and flanking sequences of a primer set (AP1 and H-T 11A) in boldface are underlined. The start codon and stop codon are shown in boldface and uppercase.](http://physiolgenomics.physiology.org/)

![Fig. 5. RNA slot blot. Total RNA (1.0 μg) from the pancreas was transferred onto a positively charged nylon membrane. The membrane was hybridized with a rat ATP5G3 probe labeled with [α-32P]dCTP (specific activity 10^5 cpm/ml). Lanes 1 and 2 (control rats) and lanes 3 and 4 (ethanol-fed rats) were hybridization signals of rat ATP5G3. Lanes 5 and 6 (control rats) and lanes 7 and 8 (ethanol-fed rats) were hybridization signals of rat 18S rRNA, which were used to normalize input RNA in each sample.](http://physiolgenomics.physiology.org/)

![ATP5G3](http://physiolgenomics.physiology.org/)

![18S rRNA](http://physiolgenomics.physiology.org/)
Liver have oxidative and nonoxidative pathways for alcohol metabolisms (1, 48). The toxic effects of acetaldehyde or the formation of fatty acid ethyl esters through these pathways may damage the mitochondria and therefore disrupt oxidative phosphorylation (32). Indeed, tissue-specific gene expression of the fatty acid ethyl esterase in pancreas and liver but not heart or brain has recently been demonstrated by our group in this model (32). Chronic ethanol consumption may depress levels of mitochondrial-encoded electron transport chain components in pancreas in a manner similar to that seen in the liver. This may lead to an adaptive upregulation of selected nuclear-encoded electron transport chain components. It is less clear how changes in the upregulation of the ATP synthase subunit 9 that are critical for mitochondrial function are upregulated in the pancreas in the present model, suggesting an adaptive process that induces transcription of the nuclear-encoded mitochondrial proteins which are required for ATP generation in the pancreas during mitochondrial injury caused by chronic ethanol consumption. The high copy number of subunit 9 required to form each ATP synthase F0 complex may have increased the likelihood that these gene transcripts were detected by differential gene expression analysis. High ATP turnover in the pancreas during synthesis of digestive enzymes stimulates mitochondrial ATP synthesis, whereas mitochondrial damage from chronic alcohol consumption likely limits the efficiency of ATP production and causes metabolic stress. Imbalances between energy demand and supply in the pancreas may increase the gene expression of the mitochondrial subunit 9 through unknown metabolic signals, resulting in an increase in transcriptional factors that complete a negative feedback loop. Although demonstration of a link between increased mRNA levels and protein levels was not performed in this study, the continuing injury to the mitochondria may also greatly enhance mitochondrial protein turnover. In this situation, increased ATP5G3 mRNA levels may be observed at the same time when protein levels and function are decreased. Thus the best measurement of the mitochondrial stress response may be the mRNA levels demonstrated in this study, followed by protein turnover assays.

Table 3. Sequence homologies of three transcripts (ATP5G1, ATP5G2, ATP5G3) encoding mitochondrial ATP synthase subunit 9 isoforms 1, 2, and 3 between human and other species

<table>
<thead>
<tr>
<th>Species</th>
<th>ATP5G1 Homology</th>
<th>GenBank</th>
<th>ATP5G2 Homology</th>
<th>GenBank</th>
<th>ATP5G3 Homology</th>
<th>GenBank</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Homo sapiens)</td>
<td>100%</td>
<td>NM_005175 (11)</td>
<td>100%</td>
<td>NM_005176 (11)</td>
<td>100%</td>
<td>U09813 (50)</td>
</tr>
<tr>
<td>(Mus musculus)</td>
<td>89%</td>
<td>NM_007506 (33, 43)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(Rattus norvegicus)</td>
<td>91%</td>
<td>NM_017311 (18)</td>
<td>86%</td>
<td>D13124 (18)</td>
<td>98%</td>
<td>AF315374 (this study)</td>
</tr>
<tr>
<td>(Ovis aries)</td>
<td>90%</td>
<td>X69904 (29)</td>
<td>91%</td>
<td>X69905 (29)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(Bos taurus)</td>
<td>91%</td>
<td>X05218 (15)</td>
<td>91%</td>
<td>X05219 (15)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Reference citations are in parentheses. NA, not available.
In summary, nuclear genes encoding mitochondrial ATP synthase subunit 9, the energy-driving motor, are upregulated at the transcriptional levels after chronic alcohol ingestion. This work demonstrates the utility of gene profile technology in identifying new genes and potential molecular mechanisms that explain altered and adaptive pathophysiological processes in the pancreas associated with alcohol abuse. Finally, it draws attention to mitochondrial injury in acinar cells during alcohol ingestion and provides a focus for future studies investigating the mechanisms whereby alcohol consumption increases the risk of alcohol-related pancreatitis.

GenBank accession number for the rat mitochondrial ATP5G3 cDNA sequence is AF315374.

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