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# Liver Galβ1,4GlcNAc α2,6-sialyltransferase is down-regulated in human alcoholics: possible cause for the appearance of asialoconjugates

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**Abstract**

Galβ1,4GlcNAc α2,6-sialyltransferase (ST6GalI) mediates the glycosylation of proteins and lipids to form functionally important glycoproteins and glycolipids in the Golgi compartment. Our previous work demonstrated that long-term ethanol feeding in rats caused a marked 59% decrease in ST6GalI activity as well as *ST6GalI* messenger RNA (mRNA) level in the liver that was due to decreased stability of the mRNA. Clinical observations show that down-regulation of *ST6GalI* gene and consequent impaired activity of ST6GalI seems to be the major cause for the appearance of asialoconjugates in the blood of long-term alcoholics. The plasma carbohydrate-deficient transferrin (CDT) and sialic acid index of plasma apolipoprotein J were also altered in the alcoholic group compared with the nondrinkers. We have now investigated how alcohol affects the gene regulation of *ST6GalI* and the possible mechanism in postmortem human liver specimens taken from nondrinkers, moderate alcohol drinkers, and heavy alcohol drinkers. Real-time polymerase chain reaction analyses of the liver RNA extract showed that *ST6GalI* mRNA level was progressively decreased by 49% in moderate drinkers ($P < .01$) and by 69% in heavy drinkers ($P < .01$) compared with nondrinkers. Western blot analysis showed that liver ST6GalI protein level was negligibly decreased in moderate drinkers but decreased by 30% ($P < .05$) in heavy drinkers compared with nondrinkers. We further demonstrated a single *ST6GalI* mRNA-binding protein complex in the normal human liver extract, which progressively decreased in the liver extracts of moderate and heavy alcohol drinkers. Thus, it is concluded that the appearance of asialoconjugates in alcoholics is possibly due to the down-regulation of *ST6GalI* gene expression.


## 1. Introduction

Sialyltransferases catalyze the terminal step in the biosyntheses of various glycoconjugates by mediating the attachment of sialic acids from cytidine-5′-monophosphate-*N*-acetylneuraminic acid to the end of the carbohydrate chains of glycoproteins and glycolipids. Carbohydrate chains of glycoproteins and glycolipids on the cell surface play important roles in biological events, such as cell-cell adhesion and communication [1-3], viral-host recognition [4], and tumor invasiveness [5]. The structures of these carbohydrate chains have been clearly established to have sialic acids as the terminal carbohydrate residue in all these glycoconjugates. Therefore, the status of sialyltransferases is critically important in both physiologic and pathologic conditions. Sialyltransferases may link the sialic acid residues to the glycoconjugate in a number of ways: either through an α2,3- or an α2,6-linkage to galactose, or through an α2,6-bond to *N*-acetylgalactosamine; or through an α2,8-bond to another sialic acid to form sialic acid chains. Different sialyl linkages are elaborated by different members in the sialyltransferase family [6,7].

Galβ1,4GlcNAc α2,6-sialyltransferase (ST6GalI) mediates the addition of α2,6–linked sialic acid to glycoproteins in the Golgi compartment. A second α2,6-sialyltransferase (ST6GalII) has been identified recently [8,9], but its expression was detected mainly in the small intestine, colon, and fetal brain [8]. Moreover, because ST6GalII is an oligosaccharide-specific enzyme that exhibits relatively low and no activities toward some glycoproteins and glycolipids,

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Table 1
Patient profiles for postmortem human liver specimens

| Group | Average age (y) | Sex ratio (M/F) | Cause of death | Daily alcohol consumption (g) |
|---|---|---|---|---|
| ND | 48.08 | 9/4 | Randomized | <14 |
| MD | 50.08 | 9/4 | Randomized | 14-42 |
| HD | 50.75 | 11/1 | Randomized | <84 |

respectively [8-11], ST6GalI remains as the major α2,6-sialyltransferase responsible for the broad synthesis of glycoproteins and glycolipids.

Clinical observations also show increased appearance of asialoconjugates in the blood of long-term alcoholics [12-14]. Because apolipoprotein J (ApoJ) has 7 times more sialic acid per mole of ApoJ, we had hypothesized that the plasma sialic acid index of plasma ApoJ (moles of sialic acid per mole of ApoJ; SIJ) would be a viable marker for long-term alcohol consumption. Accordingly, we have demonstrated that plasma SIJ is significantly decreased by 50% ($P < .001$) in human long-term alcoholics of both sexes compared with nondrinkers [14]. In our rat alcohol-feeding model, *ST6GalI* messenger RNA (mRNA) expression is reduced by as much as 59% by long-term alcohol treatment compared with the pair-fed control group in a dose-dependent manner [15,16]. We have shown that the concomitant decreased hepatic ST6GalI activity is due to its decreased synthetic rate [17], whereas the down-regulation of *ST6GalI* mRNA is due to its decreased stability [16]. In view of the pivotal role played by *ST6GalI* in alcohol-induced defective glycosylation and consequent appearance of asialoglycoconjugates in the blood, we have now investigated the gene expression of *ST6GalI* in postmortem human liver specimens taken from nondrinkers (ND), moderate alcohol drinkers (MD), and heavy alcohol drinkers (HD). The present study demonstrates that both liver ST6GalI mRNA and its protein are markedly decreased in liver specimens of heavy alcohol drinkers compared with nondrinkers, which is consistent with our previous findings in the rat model [15-17]. This down-regulation of ST6GalI may be due to the gradual loss of a cytosolic binding protein that interacts with *ST6GalI* mRNA and stabilizes it.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Tri-Reagent was purchased from Molecular Research Center (Cincinnati, OH). Primers were synthesized by Operon Technologies (Alameda, CA). Molecular biology reagents were procured from Invitrogen (Carlsbad, CA) or Bio-Rad (Hercules, CA).

### 2.2. Ethical guidelines

All human specimens collected conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the priori and also approved by the Institutional Review Board of Veterans Affairs Medical Center, Washington, DC.

### 2.3. Plasma carbohydrate-deficient transferrin and SIJ determination

For the measurement of plasma carbohydrate-deficient transferrin (CDT) and sialic acid index of plasma ApoJ (moles of sialic acid per mole of ApoJ; SIJ), 5 mL of whole blood was collected and plasma was prepared from a group of 12 male alcoholics (consuming <60 g ethanol per day) and 12 male nondrinkers. Informed consent was obtained before blood was taken individually. Plasma CDT and SIJ were determined as described by us previously [14].

### 2.4. Liver specimens

Postmortem human liver specimens (all specimen identities were kept anonymous) were purchased from Tissue Transformation Technologies (Edison, NJ) according to the following criteria:

1. Non–alcohol drinkers (ND): less than 1 alcoholic beverage per day (<14 g ethanol per day) in the past 10 years before death;
2. Moderate alcohol drinkers (MD): 1 to 3 alcoholic beverages per day (14-42 g ethanol per day) in the past 10 years before death;
3. Heavy alcohol drinkers (HD): less than 6 alcoholic beverages per day (<84 g ethanol per day).

The drinking histories of study subjects were based on clinical reports from the donor institution and the clinical classification by the hepatologist.

Patients with history of other drug use were excluded. Sex ratios in each group were biased toward men, which is comparable to a similar sex distribution in the real society of alcoholics. Average age for each group is as follows: ND, 48.08 years; MD, 50.08 years; HD, 50.75 years. Autopsy sampling was randomized for patient's cause of death (see Table 1). Autopsy was performed within 6 to 8 hours after death and autopsy specimens were promptly frozen in liquid nitrogen and stored at −80°C until analyses.

### 2.5. RNA isolation

Total RNA was isolated from each liver specimen of all groups using the Tri-Reagent (MRC, Cincinnati, OH) following the manufacturer's instructions. Adequate measures were undertaken to ensure high-quality RNA extraction from all specimens. Briefly, 500 mg of liver was homogenized in 1 mL of Tri-Reagent. The homogenate was left for 5 minutes at room temperature, then 0.2 mL of bromochloropropane (MRC) was added and the mixture was vigorously shaken for 15 seconds. The mixture was left for 15 minutes at room temperature. After centrifugation (12 000*g* for 20 minutes) at 4°C, the upper aqueous phase was carefully transferred into a sterile tube. The RNA was precipitated by addition of 0.5 mL of isopropanol and incubated at room

temperature for 5 minutes. The RNA was pelleted by centrifuging again at 12 000*g* at 4°C for 15 minutes. The precipitated RNA was washed in 70% ethanol, briefly air-dried, and then solubilized in Formazol (MRC). Total RNA concentrations were measured by absorbance reading at 260 nm on a Spectromax 190 (Molecular Devices, Sunnyvale, CA). The purity of total RNA specimen was examined by determining the $A_{260}/A_{280}$ ratio. Isolated RNA was used immediately or stored at −80°C until use.

### 2.6. Golgi fraction preparation

Golgi fractions were prepared according to a method described by Leelavathi et al [18]. All steps were performed on ice with proteinase inhibitors in all the solutions used. Briefly, 2 to 3 g of livers were homogenized in 5 volumes (wt/vol) of 0.5 mol/L sucrose made in 0.1 mol/L potassium phosphate buffer (pH 6.65) for 30 seconds with a Polytron (Glas-col, Terre Haute, IN) at 100 rpm. The homogenate was then centrifuged at 600*g* for 10 minutes at 4°C in a Beckman model J-6M centrifuge. The supernatant fraction was aspirated and 6 mL was carefully layered on top of 7 mL of 1.3 mol/L sucrose in a 15-mL tube and centrifuged at 105 000*g* for 60 minutes at 4°C. The thick membrane layer above the 1.3 mol/L sucrose interphase was aspirated and carefully layered on top of 1.1 mol/L sucrose and further centrifuged at 105 000*g* for 90 minutes. Then the Golgi membrane fraction concentrated at the interphase of 1.1 mol/L sucrose was aspirated and its purity was verified by measuring galactosyltransferase activity (92% of total liver activity in our specimen). Fifty micrograms of the Golgi fraction from each liver was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent Western blot analyses.

### 2.7. Liver cytosol extract preparation:

The liver cytosolic fraction was prepared in our laboratory as previously described [19] and was either used directly or stored at −80°C. After determining the total protein concentration of the cytosolic fraction by the Bradford method (Bio-Rad) it was generally diluted to 5 $\mu$g/$\mu$L.

### 2.8. Riboprobe template preparation

DNA template for the synthesis of mRNA probe in vitro was prepared by amplifying a 2672–base pair fragment covering the 3′-untranslated region (UTR) of h*ST6GalI* by reverse transcription-polymerase chain reaction (RT-PCR). Five micrograms of total RNA extracted from a human liver in the ND group was used in 20 $\mu$L RT reaction containing 10 pmol/L oligo(dT), 10 $\mu$mol/L 2′-deoxynucleoside-5′-triphosphate (dNTPs), and 1 U of reverse transcriptase. A typical PCR reaction mixture included 2 $\mu$L of cDNA templates from RT, 10 pmol/L of each primer, 10 $\mu$mol/L of dNTPs, 3 mmol/L of $MgCl_2$, 10× buffer, and 2 U of high-fidelity *Taq* DNA polymerase in a reaction volume of 50 $\mu$L. The PCR conditions were as follows: 2 minutes at 94°C followed by 35 cycles at 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 3 minutes. The primer pairs used were as follows: forward primer, 5′-TAATACGACTCACTATAGTTGGGAGCTATGGGACATTC-3′, and reverse primer, 5′-CTACCCAGTGTCGTCCCAGT-3′. The forward primer included a T7 RNA polymerase promoter sequence, which is underlined.

### 2.9. Phosphorus 32–labeled RNA transcript preparation

Phosphorus 32 ($^{32}$P)–labeled and unlabeled RNA probes were transcribed in vitro with T7 RNA polymerase from PCR-amplified 3′-UTR of ST6GalI according to the method described by Milligan et al [20]. Briefly, the reactions were performed in the presence of 1 $\mu$g of template in a buffer containing 2 nmol/L of [$\alpha$-$^{32}$P] ribonucleotide uridine triphosphate (800 Ci/mmol), 2.5 mmol/L of each unlabeled ribonucleotide guanidine triphosphate, ribonucleotide cytidine triphosphate, and ribonucleotide adenosine triphosphate, 40 units of ribonuclease inhibitor, and 2 U of T7 polymerase for 1 hour at 37°C. One unit of ribonuclease-free deoxyribonuclease was then added to the reaction and left at 37°C for another 30 minutes, and unincorporated nucleotides were removed by centricon-30 membrane filtration (Millipore, Billerica, MA). $^{32}$P-labeled RNA transcripts were quantified by liquid scintillation counting.

### 2.10. Quantitative real-time RT-PCR

cDNA templates for use in quantitative real time PCR were synthesized from 5 $\mu$g of total RNA by in vitro transcription in 20 $\mu$L reaction containing 0.5 $\mu$g oligo(dT), 10 $\mu$mol/L dNTPs, and 1 $\mu$L of Superscript II reverse transcriptase (Invitrogen) at 42°C for 50 minutes. Typical real-time PCR reaction mixture included the same amount of cDNA templates from RT, 10 pmol/L of each primer, 25 $\mu$L iQ SYBR Green Supermix (Bio-Rad), and sterile water in a reaction volume of 50 $\mu$L. The PCR conditions were 3 minutes at 95°C followed by 40 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The primer pairs for human ST6GalI gene were 5′-GTGGGCACAAAAACTACCAT-3′ (forward primer) and 5′-GGCTCTGGGCTCATAAACTG-3′ (reverse primer). This primer pair was first tested by regular PCR to be highly effective and specific for amplification. $\beta$-Actin was used as the standard housekeeping gene. Ratios of *ST6GalI* gene and $\beta$-actin gene expression levels were calculated by subtracting the threshold cycle number (Ct) of the target gene from the Ct of $\beta$-actin and raising 2 to the power of the negative of this difference. Ct values are defined as the number of PCR cycles at which the fluorescent signal during the PCR reaches a fixed threshold. Target gene expression is expressed relative to $\beta$-actin gene expression.

### 2.11. SDS-PAGE and Western blot analysis

Protein samples were diluted into SDS-PAGE sample buffer (50 mmol/L Tris [pH 6.8], 2% SDS, 10% glycerol,

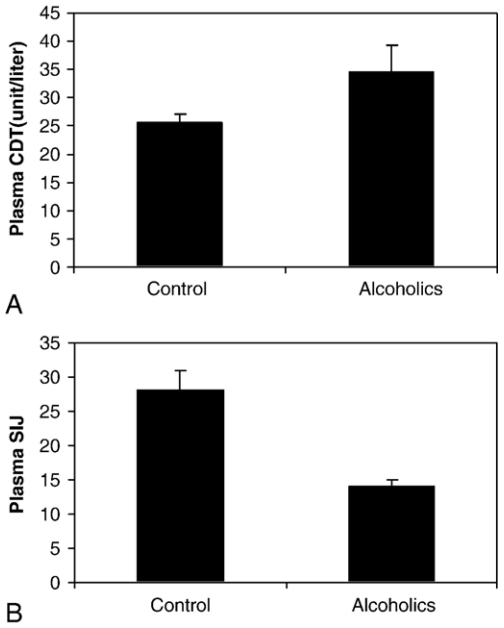


Fig. 1. Plasma CDT (A) and SIJ (B) in alcoholics and nondrinkers. A suitable aliquot of each plasma sample was analyzed for CDT and SIJ as described by us previously [14]. Plasma CDT is expressed as units per liter based on the Kabi Pharmacia Diagnostics, Uppsala, Sweden. Each value is the mean ± SEM of 12 specimens from each group.

15 mmol/L 2-mercaptoethanol, and 0.25% bromophenol blue) and boiled for 5 minutes. The specimens were elctrophoretically resolved on 4% to 15% SDS-PAGE gels. The gels were transblotted onto polyvinylidene difluoride membranes (Bio-Rad) and were processed for immunoquantification using a polyclonal anti-ST6GalI antibody (kindly provided by Dr Karen J. Colley, University of Illinois, Chicago, IL) or anti–$\beta$-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The antigen-bound primary anti-ST6GalI and anti–$\beta$-actin antibodies were detected with horseradish peroxidase–conjugated goat anti–rabbit IgG antibodies (Santa Cruz Biotechnology). Bound antibodies were visualized by enhanced chemilumiscence with reagents from Perkin Elmer (Boston, MA) and the intensity of each band was quantified with a Fluorchem 8800 densitometer (Alpha Innotech, San Leandro, CA).

### 2.12. RNA-protein electrophoretic gel mobility shift assays

$^{32}$P-labeled RNA at a concentration of 2 nmol/L was incubated with 20 $\mu$g liver cytosol proteins (this amount of protein was determined to be sufficient to visualize an RNA-protein complex in preexperiments) in a 10-$\mu$L solution containing 10 mmol/L HEPES (pH 7.5), 25 mmol/L KCl, 10% glycerol, and 1 mmol/L dithiothreitol at 30°C for 30 minutes followed by incubation with 20 U of RNase T1 (Ambion, Austin, TX) at 37°C for 10 minutes in each reaction. For competition experiments, the cytosolic fraction was incubated for 10 minutes with 100-fold molar excess of unlabeled RNA transcripts before incubation with the labeled RNA. Then 2 $\mu$L of 6× native gel loading buffer (30% glycerol, 0.025% bromophenol blue, and xylene cyanol) was added, and the RNA-protein complexes were resolved on an 8% native polyacrylamide gel in 0.5× TBE buffer (45 mmol/L Tris-HCl [pH 8.3], 45 mmol/L borate, 2.5 mmol/L EDTA). Gels were preelectrophoresed for 30 minutes at 20 mA followed by electrophoresis at 30 mA for 0.5 to 1 hour at 4°C. Gels were dried and exposed to Kodak XAR-5 film (Sigma-Aldrich, St Louis, MO) with an intensifying screen at −80°C overnight. The intensity of each band was quantified with a Fluorchem 8800 densitometer (Alpha Innotech). To ensure the comparable measurement of the bands, same numbers of specimen from each group were resolved simultaneously on the same gel; a known sample from the ND group that was previously tested to show ST6GalI-protein interaction was used as control for comparable results on separate gels.

### 2.13. Statistical analysis

Data are presented as the mean ± SEM. Statistical significance was determined by 1-way analysis of variance test followed by the Tukey test (SAS Software, SAS Institute, Cary, NC).

## 3. Results

### 3.1. Plasma CDT and SIJ in alcoholics and nondrinkers

As can be seen in Fig. 1, plasma CDT increased by 35% ($P < .05$) in the alcoholic group compared with nondrinkers (Fig. 1A), whereas plasma SIJ markedly decreased by 50% ($P < .001$) in the alcoholic group compared with the nondrinking group (Fig. 1B).

### 3.2. Liver ST6GalI mRNA level is down-regulated proportionately to the amount of alcohol consumed

*ST6GalI* mRNA levels, as determined by quantitative real-time RT-PCR, in the postmortem liver specimens of ND,

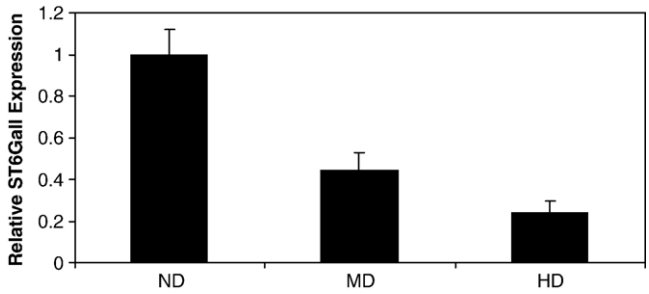


Fig. 2. Real time RT-PCR analyses of *ST6GalI* in human liver specimens. Total RNA from the ND, MD, and HD group was reverse transcribed and used in the real-time PCR. The RNA levels were normalized to the level of $\beta$-actin on an experiment-to-experiment basis. Each sample analysis was performed in triplicate independently and each bar graph represents the mean ± SEM of 12 specimens in each group with the ND group set at 1 for convenience.

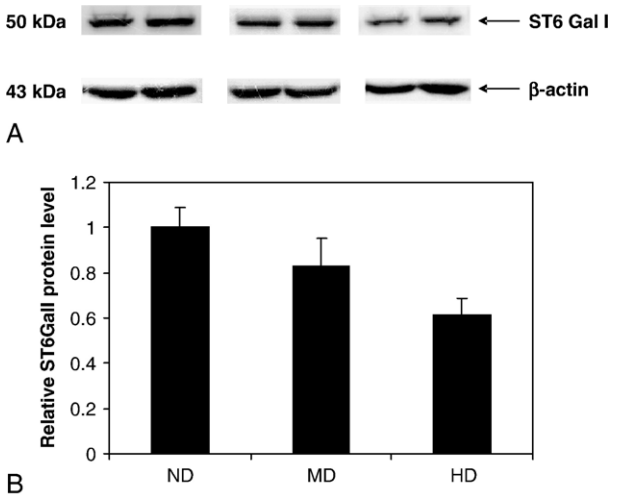


Fig. 3. Western blot analysis of *ST6GalI* in human liver specimens: 50 $\mu$g of liver Golgi fraction extracts from the ND, MB, and HD groups was subjected to Western blot analyses using the polyclonal anti-ST6GalI and anti–$\beta$-actin antibodies. Densitometry analysis of the intensity of each band was then performed. (A) Western blot of 2 representative specimens from each group. Bands on the top represent ST6GalI; bands on the bottom represent $\beta$-actin. (B) Relative intensity of ST6Gal1 protein level in each specimen was normalized to its $\beta$-actin level. The bar graphs represent the relative level of ST6GalI in each group with the ND group set at 1 for convenience. The value in each group is the mean ± SEM of 12 specimens.

MD, and HD groups are shown in Fig. 2. ST6GalI mRNA level was decreased on an average by 49% ($P < .01$) and by 69% ($P < .01$) in moderate and heavy alcohol drinkers, respectively, compared with the nondrinkers.

### *3.3. Down-regulation of ST6GalI mRNA by alcohol concomitant with the decrease in ST6GalI protein*

Because any change in a given mRNA level does not always reflect in a corresponding change in its protein level or enzyme activity, it is necessary to determine the protein level to give a meaningful interpretation of our above observations. Therefore, Western blot analyses of the liver Golgi extracts from postmortem liver specimens of the 3 experimental groups were performed using anti-ST6GalI antibody. The results are shown in Fig. 3. The hepatic ST6GalI protein level relative to that of $\beta$ actin was not significantly decreased (10%, $P = .4$) in the moderate drinkers, whereas it was decreased by 30% ($P < .05$) in heavy alcohol drinkers compared with nondrinkers. These results are consistent with our finding of the progressive down-regulation of *ST6GalI* mRNA with the magnitude of alcohol consumption (Fig. 1).

### *3.4. The level of the cytosolic binding protein that protects ST6GalI mRNA stability*

Recently, we clearly characterized a cytosolic protein that stabilizes rat *ST6GalI* mRNA by specifically interacting with its 3′-UTR domain, but this binding protein was completely depleted after long-term alcohol consumption in a rat model [21]. To find out whether a similar regulatory mechanism operates in the livers of human alcoholics, RNA-protein electrophoretic gel mobility shift assay (EMSA) was performed on all 36 postmortem liver specimens. Prior experiments were carried out to obtain the optimal amount of cytosol protein to be used and to verify that RNA-protein interaction is specific for ST6GalI. Two representative samples from each ND, MD, and HD group are shown in Fig. 4A. A single cytosolic protein complex with the human *ST6GalI* mRNA was evident in the liver extracts of nondrinkers. When quantitative measurement was performed on all specimens in each group with a known standard, the level of the cytosol protein gradually decreased in the liver extracts from moderate ($P < .05$) and heavy alcohol drinkers ($P < .01$) (Fig. 4B).

## 4. Discussion

To investigate whether alcohol consumption affects the *ST6GalI* gene regulation in postmortem human livers, we determined ST6GalI mRNA levels by quantitative real-time RT-PCR in all the liver specimens from the 3 groups. We show for the first time in humans that moderate drinking down-regulates *ST6GalI* mRNA level by as much

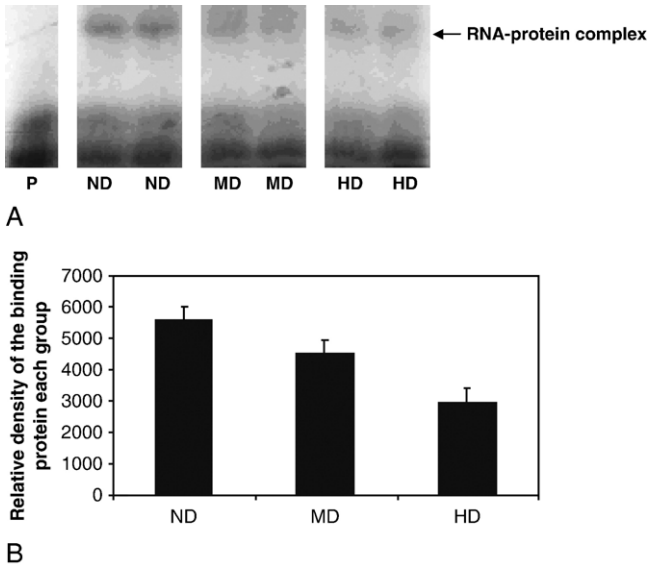


Fig. 4. RNA-protein EMSA. $^{32}$P-labeled RNA at a concentration of 2 nmol/L was incubated with 20 $\mu$g of cytosolic proteins from each liver sample and the RNA-protein complexes were resolved on an 8% native polyacrylamide gel in 0.5× TBE buffer. The gel was then dried and exposed to XAR film with an intensifying screen at −80°C overnight. Two representative specimens from each group are shown in (A). The arrow indicates the RNA-protein complex; Lane P represents RNA probe without the cytosolic protein. (B) Average density of 12 specimens in each group. The value in each group is the mean ± SEM of 12 specimens. The RNA-protein complex was progressively decreased in both MD and HD groups compared with the ND group.

as 49% ($P < .01$), and heavy drinking further diminishes its level by 69% ($P < .01$) (Fig. 2.). This is further confirmed by concomitant changes in ST6GalI protein (Fig. 3). Similar alterations in plasma CDT and SIJ in the alcoholic group compared with the nondrinkers (Fig. 1) fully support our concept that the marked down-regulation of *ST6GalI* mRNA in the liver specimens of alcoholics may be responsible for the appearance of these asialoglycoconjugates in alcoholics. Unfortunately, because the blood specimens were not collected in these postmortem cases, we could not measure plasma CDT and SIJ, although we do demonstrate marked decrease in plasma SIJ in alcoholic patients compared with nondrinkers (Fig. 1A and B). At the same time, because hepatic *ST6GalI* mRNA has been reported to be unaltered or up-regulated in nonalcoholic or neoplastic liver diseases [22-24], liver disease could not be the cause for the down-regulation of *ST6GalI* mRNA in the livers of alcoholics found in the present study. Thus, our data clearly seem to support the concept that alcohol consumption per se, but not the liver disease, may be specifically responsible for *ST6GalI* mRNA down-regulation.

These results are consistent with our earlier findings in an animal model in which we previously showed that long-term ethanol exposure causes impaired expression of ST6GalI gene [16] accompanied by corresponding changes in plasma CDT and SIJ in chronic alcohol-fed animals [14]. In that model, we further demonstrated that chronic alcohol treatment caused decreased ST6GalI protein synthetic rate [17] because of decreased stability of *ST6GalI* mRNA without affecting its transcription rate [16]. We have recently partially characterized a liver cytosolic protein that specifically interacts with *ST6GalI* mRNA to stabilize it [21]. That the level of this binding protein is depleted in the liver specimens of alcoholics (Fig. 3) strongly supports our concept that the down-regulation of *ST6GalI* gene may be due to the loss of this mRNA stabilizing protein, as we have shown in the animal system [21]. Thus, the possible mechanism(s) by which short-term ethanol exposure down-regulates *ST6GalI* gene in human liver is mediated via the destabilization of its mRNA by depleting a specific cytosol-binding protein that interacts with its 3′-UTR domain.

Clinical observations also show increased appearance of asialoconjugates in the blood of long-term alcoholics [12-14]. Thus, if the specificity of ethanol exposure on this gene is established, it may form the basis for the appearance of asialoconjugates in blood of human alcoholics that can serve as viable biomarkers. Second, sialic acids and their derivatives are ubiquitous at the terminal positions of oligosaccharides of glycoproteins and glycolipids in mammalian cells and their roles have been implicated in various biological phenomena, such as cell proliferation, differentiation, signal transduction, cell surface interactions, cell-cell communication and adhesion or even viral-host recognition, or tumor invasiveness [1,25,26].

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## References

[1] Kelm S, Schauer R. Sialic acids in molecular and cellular interactions. Int Rev Cytol 1997;175:137-240.
[2] Pilatte Y, Bignon J, Lambre CR. Sialic acids as important molecules in the regulation of the immune systems: pathophysiological implications of sialidases in immunity. Glycobiology 1993;3:201-18.
[3] Lin S, Kemmner W, Grigull S, Schlag PM. Cell surface alpha 2,6 sialylation affects adhesion of breast carcinoma cells. Exp Cell Res 2002;276:101-10.
[4] Gagneux P, Cheriyan M, Hurtado-Ziola N, van der Linden EC, Anderson D, McClure H, et al. Human-specific regulation of alpha2-6–linked sialic acids. J Biol Chem 2003;278:48245-50.
[5] Zhu Y, Srivatana U, Ullah A, Gagneja H, Berenson CS, Lance P. Suppression of a sialyltransferase by antisense DNA reduces invasiveness of human colon cancer cells in vitro. Biochim Biophys Acta 2001;1536:148-60.
[6] Harduin-Lepers A, Vallejo-Ruiz V, Krzewinski-Recchi MA, Samyn-Petit B, Julien S, Delannoy P. The human sialyltransferase family. Biochimie 2001;83:727-37.
[7] Tsuji S. Molecular cloning and functional analysis of sialyltransferases. J Biochem (Tokyo) 1996;120:1-13.
[8] Takashima S, Tsuji S, Tsujimoto M. Characterization of the second type of human $\beta$-galactoside $\alpha$2,6-sialyltransferase (ST6Gal II), which sialylates Gal$\beta$1,4GlcNAc structures on oligosaccharides preferentially. Genomic analysis of human sialyltransferase genes. J Biol Chem 2002;277:45719-28.
[9] Krzewinski-Recchi MA, Julien S, Juliant S, Teintenier-Lelievre M, Samyn-Petit B, Montiel MD, et al. Identification and functional expression of a second human beta-galactoside alpha2,6-sialyltransferase, ST6Gal II. Eur J Biochem 2003;270:950-61.
[10] Appenheimer MM, Huang RY, Chandrasekaran EV, Dalziel M, Hu YP, Soloway PD, et al. Biologic contribution of P1 promoter-mediated expression of ST6GalI sialyltransferase. Glycobiology 2003;13:591-600.
[11] Takashima S, Tsuji S, Tsujimoto M. Comparison of the enzymatic properties of mouse beta-galactoside alpha2,6-sialyltransferases, ST6GalI and II. J Biochem (Tokyo) 2003;134:287-96.
[12] Stibler H, Borg S. Evidence of a reduced sialic acid content in serum transferrin in male alcoholics. Alcohol Clin Exp Res 1981;5:545-9.
[13] Tsutsumi M, Wang JS, Takada A. Microheterogeneity of serum glycoproteins in alcoholics: is desialo-transferrin the marker of chronic alcohol drinking or alcoholic liver injury? Alcohol Clin Exp Res 1994;18:392-7.
[14] Ghosh P, Hale EA, Lakshman MR. Plasma sialic acid index of apolipoprotein J (SIJ): a new alcohol intake marker. Alcohol 2001;25:173-9.
[15] Rao MN, Lakshman MR. Chronic ethanol downregulates 2,6-sialyltransferase and 2,3-sialyltransferase mRNAs in rat liver. Alcohol Clin Exp Res 1997;21:348-51.
[16] Rao MN, Lakshman MR. Chronic ethanol consumption leads to destabilization of rat liver 2,6-sialyltransferase mRNA. Metabolism 1999;48:797-803.
[17] Ghosh P, Liu QH, Lakshman MR. Long-term ethanol exposure impairs glycosylation of both N- and O-glycosylated proteins in rat liver. Metabolism 1995;44:890-8.
[18] Leelavathi DE, Estes LW, Feingold DS, Lombardi B. Isolation of a Golgi-rich fraction from rat liver. Biochim Biophys Acta 1970;211:124-38.

[19] Marmillot P, Rao MN, Lakshman MR. Chronic ethanol exposure in rats affects rabs-dependent hepatic trafficking of apolipoprotein E and transferrin. Alcohol 2001;25:195-200.

[20] Milligan JF, Groebe DR, Witherell GW, Uhlenbeck OC. Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. Nucleic Acids Res 1987;15:8783-98.

[21] Petretti T, Kemmner W, Schulze B, Schlag PM. Altered mRNA expression of glycosyltransferases in human colorectal carcinomas and liver metastases. Gut 2000;46:359-66.

[22] Wang PH, Lee WL, Lee YR, Juang CM, Chen YJ, Chao HT, et al. Enhanced expression of alpha 2,6-sialyltransferase ST6GalI in cervical squamous cell carcinoma. Gynecol Oncol 2003;89:395-401.

[23] Dall'Olio F, Chiricolo M, D'Errico A, Gruppioni E, Altimari A, Fiorentino M, et al. Expression of beta-galactoside alpha2,6 sialyltransferase and of alpha2,6-sialylated glycoconjugates in normal human liver, hepatocarcinoma, and cirrhosis. Glycobiology 2004;14:39-49.

[24] Garige M, Gong M, Lakshman MR. Ethanol destabilizes liver ST6Gal l mRNA by depleting a 3′-untranslated region specific binding protein. J Pharmacol Exp Ther 2006;318:1076-82.

[25] Schauer R. Sialic acids and their role as biological masks. Trends Biochem Sci 1985;10:357-60.

[26] Hakomori S. Structure and function of sphingoglycolipids in transmembrane signalling and cell-cell interactions. Biochem Soc Trans 1993;21:583-95.