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Novel serum biomarkers for detection of excessive alcohol use

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Abstract

Objectives—Construct interview that correctly identifies those with alcohol use disorder have limitation, especially when the subjects are motivated to minimize the magnitude of drinking behavior. Current laboratory tests to detect excessive alcohol consumption are limited by marginal sensitivity/specificity. Excessive drinking has been shown to affect several organ systems; which may be reflected in changes in quantity of plasma proteins. Our aim was to employ novel proteomic analyses to identify potential markers for excessive alcohol use.

METHODS—A prospective case-control study that included 39 controls and 54 excessive drinkers (discovery cohort). The serum proteomic analyses in these subjects were performed and the results were tested in the verification cohort (40 controls and 40 excessive drinkers).

RESULTS—Using the appropriate cutoff and confirmation with ELISA, we identified 4 proteins which were significantly elevated in the serum of excessive drinkers; AT-rich interactive domain-containing protein 4B (ARID4B), Phosphatidylcholine-sterol acyltransferase (LCAT), Hepatocyte growth factor-like protein (MST1), and ADP-ribosylation factor 6 (ARL6). The performance of the conventional markers (AST, ALT, GGT, %CDT, and MCV) discriminating between excessive alcohol use and controls had an area under the curve (AUC) ranging from 0.21 (ALT) to 0.67 (MCV). The AUC of these novel proteins showed the improvement in the detection of excessive drinkers compared to conventional lab tests, ranging from 0.73 (for ARID4B) to 0.86 (for ARL6).

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Conflicts of interest: None

CONCLUSIONS—We have identified four novel proteins that can discern subjects with excessive alcohol use. Further studies are needed to determine the clinical implications of these markers to detect excessive alcohol use and confirm abstinence.

Keywords

Proteomics; serum markers; excessive alcohol use; LC/MS

INTRODUCTION

At present, laboratory markers to detect recent excessive alcohol consumption (Bearer, C. F., Bailey, S. M., & Hoek, J. B. 2010) are limited by marginal sensitivity and specificity, uncertain interval of detection and/or considerable cost. Direct measurement of alcohol concentration in blood/urine samples is not useful as it is only present for a short time after drinking cessation, and thus does not provide information more than a few hours beyond the most recent period of alcohol use (Bearer, C. F. et al. 2010). The plasma levels of enzymes expressed in the liver, such as gamma glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the mean corpuscular volume of erythrocytes (MCV) are among the commonly used markers to identify chronic alcohol use (Liangpunsakul, S., Qi, R., Crabb, D. W., & Witzmann, F. 2010). However, in our recent study (Liangpunsakul, S. et al. 2010), the ability of these markers to determine the levels of alcohol consumption in the preceeding month revealed low sensitivities. Although %CDT, a form of the serum iron carrying protein transferrin with altered carbohydrate composition, is a more specific marker for identifying chronic excessive alcohol use and monitoring abstinence, it does not have the desired sensitivity and specificity (Golka, K., et al. 2004). Substantial efforts have been made to construct interview formats that correctly identify those with alcohol use disorder, such as AUDIT-C (Hawkins, E. J., et al. 2010), CAGE (Skogen, J. C., et al 2011), or reports from collateral family who interact with the subject (Whitford, J. L., et al 2009). Limitations of this approach are most apparent in cases where individuals are motivated to deny or minimize the magnitude of drinking behavior to mitigate personal ramifications (Freeman, W. M. & Vrana, K. E. 2010).

Analysis of serum proteins offer a promising approach to quantifiable estimation of recent excessive alcohol consumption. Immoderate drinking has been shown to affect several organ systems; which may be reflected in changes in quantity or quality of constituent or novel plasma proteins or protein fragments. Organ/tissue-specific proteins may be released into the blood stream when cells are injured by alcohol, or when systemic changes are induced by alcohol. In addition, because ethanol metabolism generates the highly reactive protein modifying reagent acetaldehyde, acetaldehyde-protein adducts have been identified in many laboratories (Conduah Birt, J. E., Shuker, D. E., & Farmer, P. B. 1998; Niemela, O. 1999; Roy, H. K., Gulizia, J. M., Karolski, W. J., Ratashak, A., Sorrell, M. F., & Tuma, D. 2002).

The general approach to serum/plasma-based biomarker development has been to remove the most abundant proteins to improve the resolution of low abundance protein candidates. However, it has become clear after several years of pursuing this strategy that many of the very low-abundance peptide and protein markers are either present in undetectable levels or

absent as a result of being excreted by the urinary system or bound to high-abundance carrier proteins in the plasma (Nomura, F., et al. 2004; Stibler, H., Borg, S., & Allgulander, C. 1979).

Because the low molecular weight portion of the human blood proteome is comprised of peptides and protein fragments, it has attracted significant interest, yet remains relatively un-mined. Binding to high-abundance carrier proteins such as albumin prevents excretion by the kidneys, extending the half-lives of these potential disease biomarkers. A blood sample preparation approach that takes advantage of this inherent biological enrichment of disease-associated low molecular weight biomarkers has been coupled with high resolution mass spectrometry and discriminant pattern analysis. The combination is a powerful tool for diagnostics and population screening and has been used successfully to investigate biomarkers of ovarian cancer (Fisher, W. G., et al. 2007; Petricoin, E. F., et al. 2002), and Alzheimer's disease (Lopez, M. F., et al. 2005). In this project, we employed that approach to identify potential markers for excessive alcohol use (Lai, X., et al. 2009; Liangpunsakul, S., et al 2009). We hypothesized that the serum proteome in excessive alcohol use subjects qualitatively and quantitatively differs from the proteome of those who drink only in moderation.

Methods

The first step in our strategy to test our working hypothesis employed quantitative proteomics based on label-free quantitative mass spectrometry on serum samples obtained from a discovery cohort. We then tested the validity of the findings in a verification cohort of similar composition, and compared the results to assay of the enzymes currently used for detection of excessive drinking.

The outline of the study is shown in Figure 1. For the discovery cohort, 54 subjects with history of alcohol use disorder (AUD) who were admitted for alcohol rehabilitation at Fairbanks Drug and Alcohol Treatment Center (Indianapolis, IN) were recruited. They all met the criteria for AUD (defined by the DSM IV criteria) and 'excessive drinking'; the latter defined by NIH/NIAAA as men who drink more than 4 standard drinks in a day (or more than 14 per week) and women who drink more than 3 standard drinks in a day (or more than 7 per week). Forty nine non-excessive drinkers were recruited from Roudebush Veterans Administration Medical Center (RVAMC) in Indianapolis, Indiana. The verification cohorted comprised 40 subjects with AUD (From Fairbanks) and 40 non-excessive drinker controls (from RVAMC). The inclusion criteria required subjects to be at least 21 years of age or older and be able to provide informed consent. Subjects were excluded if they had active and serious medical diseases (such as congestive heart failure, chronic obstructive pulmonary disease, cancer, uncontrolled diabetes, and chronic renal failure) at the time of screening; had history of any systemic infection within 4 weeks prior to the study; or had history of recent major surgeries within the past 3 months. The study design and protocol were approved by the Institutional Review Board. Written informed consent was obtained from each participant.

Data Collection/clinical evaluation

Participants completed a self-administered questionnaire such as demographic data and AUDIT-C. The Time Line Follow-Back (TLFB) questionnaire was used to determine the amount of alcohol consumption over the 30-day period before the study date. It was administered in person by trained study coordinators who reviewed the instructions with the subjects prior to administering the questionnaire. The TLFB offers a retrospective report of daily alcohol consumption over the past 30 days; drinks per drinking occasion, and pattern of drinking can be computed (Sobell, L. C., Agrawal, S., Sobell, M. B., Leo, G. I., Young, L. J., Cunningham, J. A. et al. 2003; Sobell, L. C., Sobell, M. B., Riley, D. M., Schuller, R., Pavan, D. S., Cancilla, A. et al. 1988; Vakili, S., Sobell, L. C., Sobell, M. B., Simco, E. R., & Agrawal, S. 2008). In addition, blood samples were obtained for assay of commonly used markers to identify chronic alcohol use (such as GGT, CDT, AST, and ALT and MCV)

Sample preparation, mass spectrometry, protein identification and relative quantification

See the supplementary materials and methods section for more detail.

Enzyme-linked immunosorbent assay (ELISA)

To validate the groups' differential protein levels identified by quantitative mass spectrometry ELISA was carried out using commercially available kits for proteins of specific interest. See the supplementary materials and methods section for more detail.

Statistical analysis

Basic descriptive statistics, including mean, standard deviations (S.D), and percentages were used to characterize the study subjects. Appropriate comparison tests including chi-square test and student t-test were used for comparison between groups for categorical and continuous variables, respectively (Figure 1). For ELISA results, mean (±S.D) of the serum level of the proteins of interest were calculated, and used to construct a diagnostic model. For verification cohort, ROC curves of each protein levels predicting the excessive alcohol consumption and the area under curve (AUC) were obtained. Sensitivity, and specificity analyses of each protein of interest were calculated for the verification cohort. We also performed principle component analysis (PCA) to identify most influencial biomarker or the combination with the large impact on detecting excessive alcohol use. All analyses were conducted with SPSS (Chicago, IL, USA).

Results

Clinical characteristics of discovery and verification cohorts

The clinical characteristics are presented in Table 1. In both discovery and verification cohorts, there were no statistically significant differences in age, gender, race and BMI between controls and excessive drinkers, but 30-day drinking histories were quite different. *In the discovery cohort*, excessive drinkers had higher AUDIT scores (29 vs. 4), greater total standard drinks in the past 30 days (335 vs. 15 drinks), higher average drinks per drinking day (15.2 vs 2.2 drinks), and a higher number of drinking days in the past month (24 vs. 5 days), when compared to controls. They had significantly higher levels of serum AST, ALT,

GGT, %CDT, and MCV. The patterns of alcohol consumption based on TLFB in the verification cohort were similar to those in the discovery cohort with elevated levels of AST, ALT, GGT, %CDT, and MCV in excessive drinkers compared to controls.

Protein identification

In the discovery cohort, proteomic analysis identified and quantified 602 unique proteins. A complete list of these proteins can be found in Supplemental Table 1. As shown in Fig 2A, these proteins were involved in several cellular functions. Of these 602 proteins, we demonstrated that 51 proteins had the potential to separate subjects with excessive alcohol use from controls and 47 were elevated in excessive drinkers (using cut-off p-value at 0.01, Fig 2B–2D, Supplemental Table 1). The informative proteins were involved in inflammatory responses, cellular organization, enzymes, and immune responses (Fig 2C)

Next, we considered the ratio of > 1.8, comparing the detected levels of protein between groups in the discovery cohort, as the cutoff value to designate significant differences in the protein expression, and found 8 proteins which met this criterion: AT-rich interactive domain-containing protein 4B, ETS domain-containing transcription factor ERF, Actin-like protein 6A, Immunoglobulin lambda, Phosphatidylcholine-sterol acyltransferase, Intercellular adhesion molecule 2, Hepatocyte growth factor-like protein, and ADP-ribosylation factor 6 (Figs 2D and 3). To confirm the findings from the LC/MS, the confirmatory ELISA assays were performed in the serum samples of subjects in the discovery cohort, and we found that the serum levels of the following 4 proteins were significantly elevated in the serum of excessive drinkers relative to controls: AT-rich interactive domain-containing protein 4B, Phosphatidylcholine-sterol acyltransferase, Hepatocyte growth factor-like protein, and ADP-ribosylation factor 6.

Verifications of the novel proteins as the candidates for biomarker

To validate the results from the discovery cohort, we compared the serum levels of AT-rich interactive domain-containing protein 4B, Phosphatidylcholine-sterol acyltransferase, Hepatocyte growth factor-like protein, and ADP-ribosylation factor 6 between the 2 goups in the verification cohort. The results of the ELISA for these 4 proteins are shown in Figure 4; the serum levels of these four proteins were significantly higher in excessive drinkers compared to controls, confirming the results from the discovery cohort.

Performance of the new novel proteins in the differential diagnosis of excessive drinkers from controls in comparison to the conventional markers

The ROC (receiver operating characteristic) area under the curve (AUC) values, sensitivity, and specificity of the new novel proteins in the verification cohort appear in Table 2, and are compared to those indices for the conventional markers commonly used to screen for excessive alcohol use. Fig 5 shows the ROC curves for these markers. The ROC of the conventional markers discriminating between excessvie alcohol use and controls had an AUC ranging from 0.21 (for serum ALT) to 0.67 (for MCV). Using the thresholds reported in Table 2, the conventional tests were diagnostic of excessive alcohol use with 76% sensitivity and 71% specificity (for GGT), 85% sensitivity and 67% specificity (for AST), 75% sensitivity and 91% specificity (for ALT), 85% sensitivity and 85% specificity (for

MCV), and 82% sensitivity and 86% specificity (for %CDT). In comparison, the new markers demonstrated superior discrimination to distinguish excessive drinkers from controls when compared to conventional markers. The sensitivity was 97% (for AT-rich interactive domain containing protein 4B and ADP-ribosylation factor 6), 95% (for Phosphatidylcholine-sterol acyltransferase), and 90% (for hepatocyte growth factor like protein) (Table 2). The ROC curves of these new novel proteins showed the improvement in the detection of excessive drinkers compared to conventional lab tests (Table 2 and Fig 5, $p < 0.05$ for the AUC of the new markers compared to each of the conventional marker). The AUC ranged from 0.73 (for AT-rich interactive domain containing protein 4B) to 0.86 (for ADP-ribosylation factor 6).

PCA were carried out to identify potential linear combination of the conventional and the new markers that accurately predict excessive alcohol consumption using the verification cohort. As shown in Tables 3 and 4, the first PCA accounted for ~ 97.8% of the variation of the dataset. PCA analysis also showed the levels of ADP-ribosylation factor 6 had the loading for the first principal component of 0.998 (Tabe 4); while the loading for other markers were less than 0.05. Our data indicated that ADP-ribosylation factor 6 had the best ability to discern subjects with excessive alcohol use, when using alone and compared to other conventional/new markers.

Correlations between the levels of conventional lab tests/the new markers and the level of alcohol consumption during the past 30 days

We computed the correlations between the levels of the conventional enzyme assays and the levels of alcohol consumption measured by the TLFB during the last 30 days for the verification cohort (Fig 6): GGT ($r = 0.3$), AST ($r = 0.3$), MCV ($r = 0.3$) and %CDT ($r = 0.2$), respectively. The levels of ALT, on the other hand, had no significant correlation with intensity of of recent alcohol consumption in the last 30 days as measured by TLFB. We also computed the same correlations for the new markers; these were comparable to the conventional assays, with the notable exception that phosphatidylcholine-sterol acyltransferase and ADP-ribosylation factor 6 had $r = 0.47$ ($p<0.0001$), accounting for twice the variance in the data set as did the best conventional marker, MCV.

DISCUSSION

If the sensitivity and specificity of serum markers to identify subjects with excessive recent alcohol use can be improved, then clinical care would be enhanced. The sensitive biomarkers would confirm self-report of alcohol consumption, but also provide results from an objective biochemical test to help physicians motivate patients to moderate or stop drinking, and would provide objective measures of progress towards that goal. Our study employed the proteomic method from serum samples and identified 4 novel proteins that appear to provide that improved performance, compared to conventional measures.

Based on the assumption that organ/tissue-specific proteins are released into the blood stream when cells are injured by alcohol, or when systemic changes are induced by alcohol, we sought to identify carrier-bound proteins that showed significant alterations in subjects with excessive alcohol use when compared to controls. These proteins we identified are

involved in inflammatory responses, cellular organization, enzymatic processes, protein transportation, and cell proliferation. These proteins are AT-rich interactive domain-containing protein 4B, phosphatidylcholine-sterol acyltransferase, hepatocyte growth factor-like protein, and ADP-ribosylation factor 6.

Our discovery of significantly increased serum expression of these 4 proteins in excessive drinkers were confirmed by consistent findings in the verification cohort. Our newly identified proteins performed better (according to the AUC of the ROC results) than the conventioinal laboratory tests routinely used to screen for excessive alcohol use. The AUC for the new markers ranged from 0.73–0.86 compared to 0.21–0.67 (for conventional markers). Though in the PCA, we found that ADP-ribosylation factor 6 was the best marker to use to detect subjects with exceesive alcohol use, we believe that this finding needs future validation in the larger sample size with sufficient sample calculation to achive the optimal power.

We observe that these newly identified proteins are mechanistically linked to alcohol consumption and metabolism. AT-rich interactive domain-containing protein 4B, encoded by the *ARID4B* gene, is a subunit of the histone deacetylase-dependent SIN3A transcriptional corepressor complex (Wu, R. C., Jiang, M., Beaudet, A. L., & Wu, M. Y. 2013). This protein possess several cellular function including proliferation, differentiation, and apoptosis; all of which are perturbed by alcohol (Sutherland, G. T., et al. 2013; Szabo, G. & Mandrekar, P. 2009). Phosphatidylcholine-sterol acyltransferase is an enzyme that catalyzes the reaction between phosphatidylcholine and sterol (Ng, D. S. 2004); participating in glycerophospholipid metabolism and normally secreted from the liver into circulation (Ng, D. S. 2004). This functionality is in accord with our previous findings of alterations in the enzyme's activity and an increase in serum phospholipids in ethanol-fed mice, compared to controls, and that several of the associated lipids are under the control of phosphatidylcholine-sterol acyltransferase (Zhao, Z., et al 2011). One Japanese study also found the positive correlation between serum phosphatidylcholine-sterol acyltransferase and chronic alcohol use (Goto, A., Sasai, K., Suzuki, S., Fukutomi, T., Ito, S., Matsushita, T. et al. 2003). Hepatocyte growth factor-like protein is an inflammatory cytokine, and can activate macrophages (similar to the effect of alcohol) (Bezerra, J. A., et al 1998). ADP-ribosylation factor 6 is a member of ADP Ribosylation Factor family of GTP-binding proteins (Donaldson, J. G. & Jackson, C. L. 2011). It is a major regulator of vesicle biogenesis in intracellular traffic (Donaldson, J. G. et al. 2011). Cells with increased ADP-ribosylation factor-like protein were found to have increased protein phosphatase 2A activity (Beghin, A., et al. 2009), an enzyme which we have shown to be activated by alcohol (Liangpunsakul, S., et al 2012).

The strengths of our study are the relatively large number of subjects in controls and those with excessive alcohol use in our discovery and verification cohorts. Our proteomic analysis platform enabled us to identify the large numbers of serum proteins, significantly elevated in the serum of subjects with excessive alcohol use, to enable selection of potential biomarker candidates. Lastly, our results were verified in a separate group of subjects by demonstrating the results were consistent with those in the discovery cohort.

One limitation of our study is that we do not yet know the usefulness and efficacy of these new markers for follow-up and for confirming a return to moderate drinking/abstinence even though we observed a correlation between the levels of each new marker and the amount of alcohol consumed in the last 30 days. A future study to determine the kinetics of these proteins as a function of the amount of alcohol consumed over time is needed. As a proof of concept, for example, we speculate that serum levels of these markers would remain elevated for some time if someone who had been drinking in excess stopped abruptly, but then steadily decline to normal over several weeks.

In summary, we have identified four newly diagnostic proteins from serum that demonstrate the ability to discern subjects with recent, excessive alcohol use from controls. Further studies are needed to confirm and determine the clinical implications of these new markers to detect excessive alcohol use and confirm moderation or abstinence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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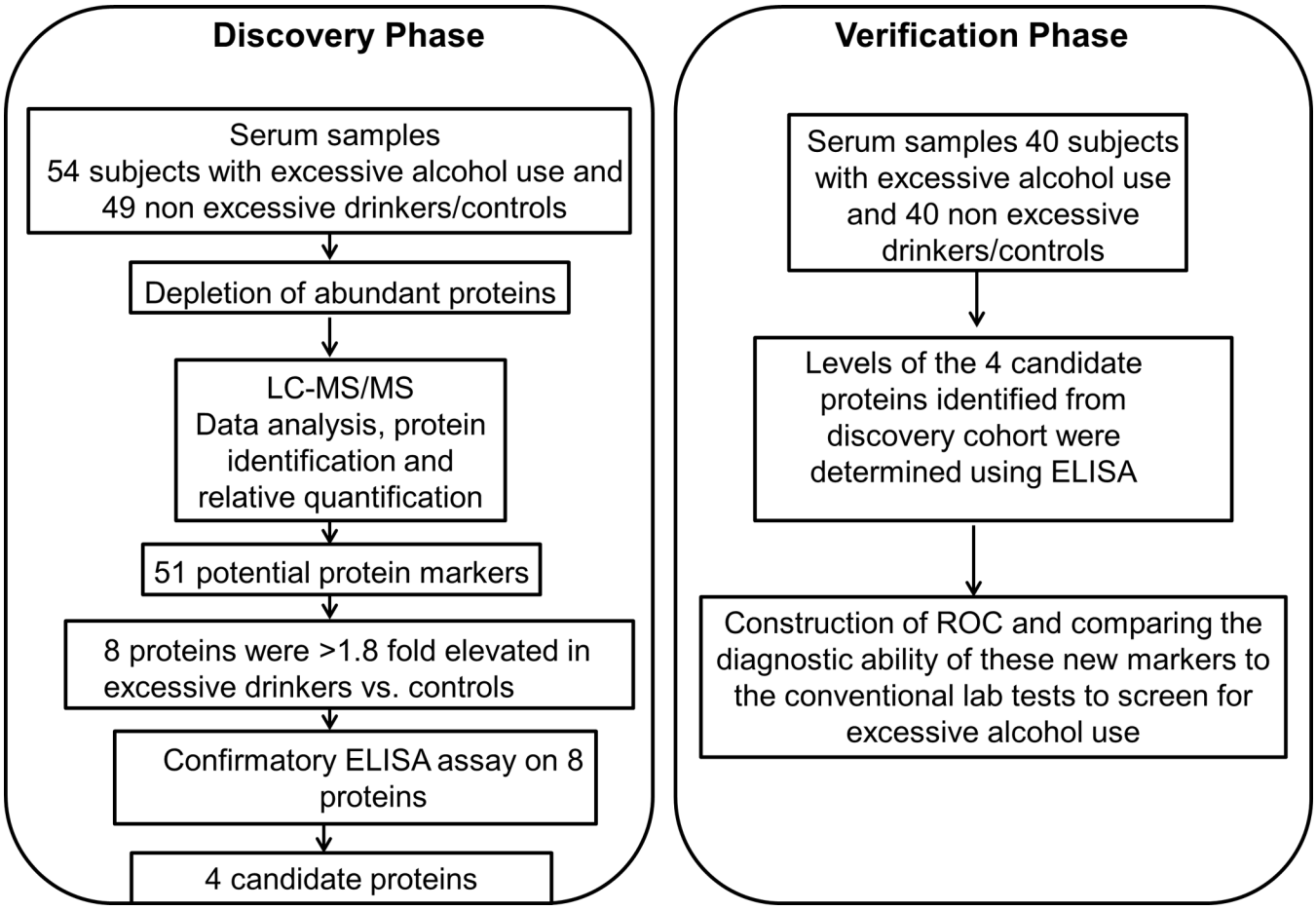


Fig 1.

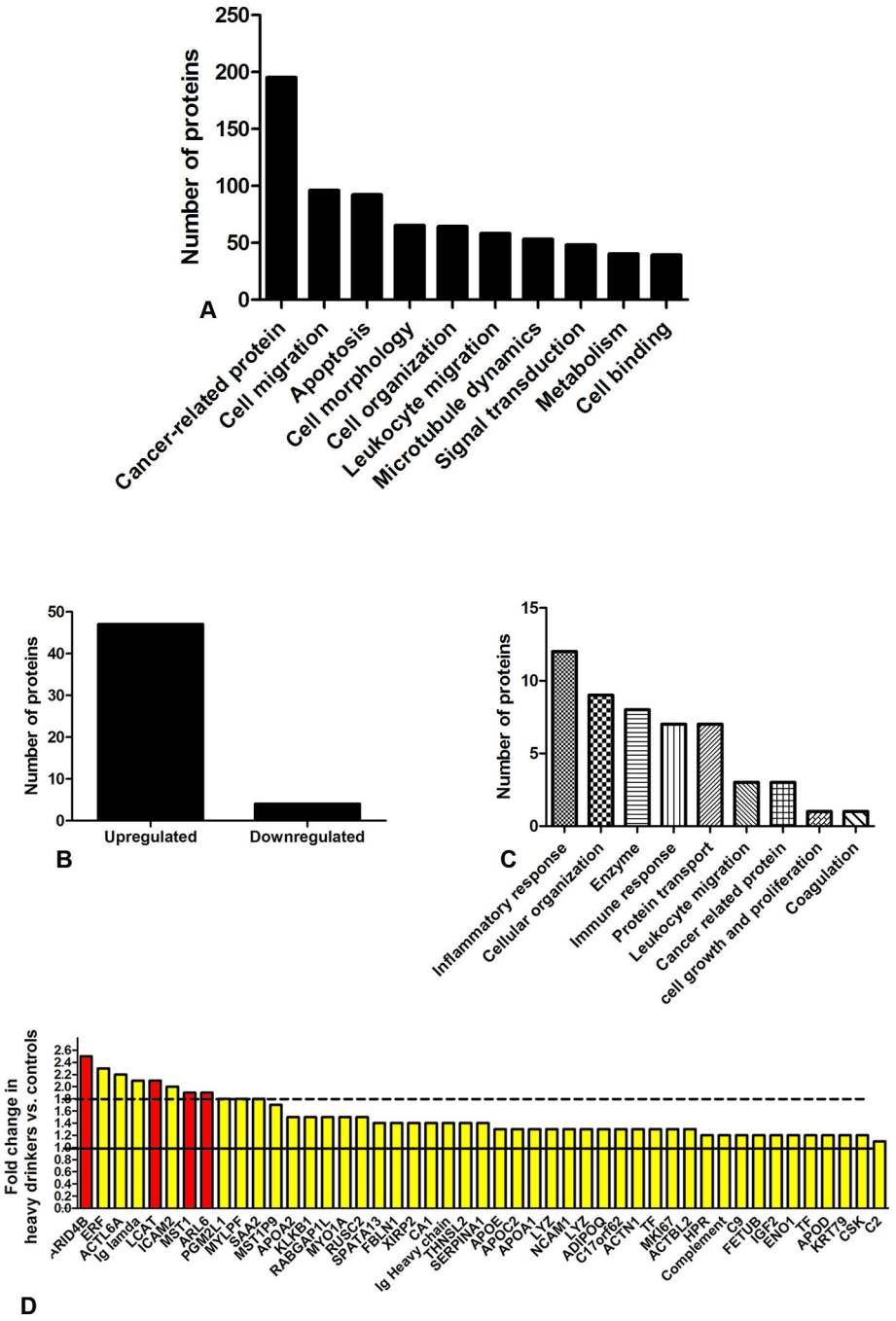


Fig 2.

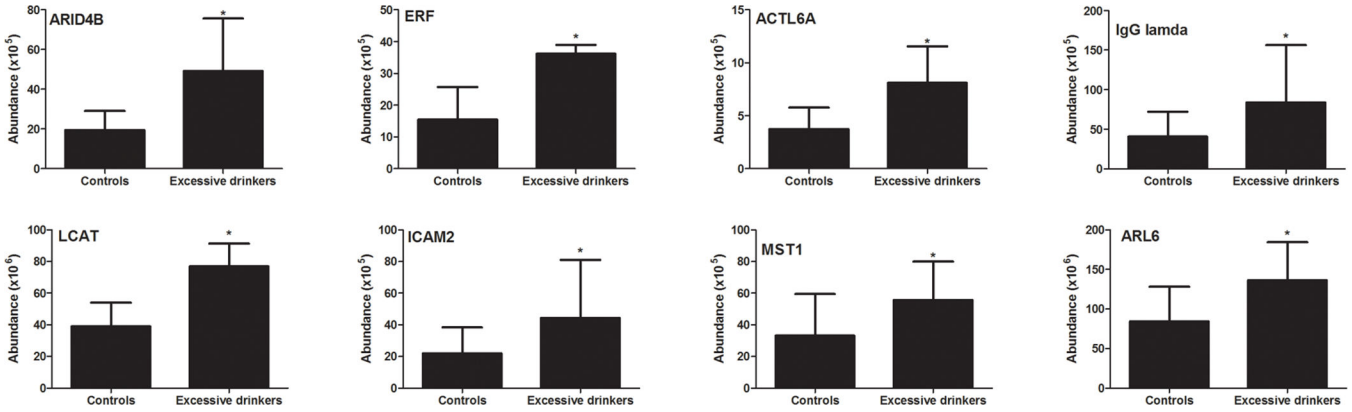
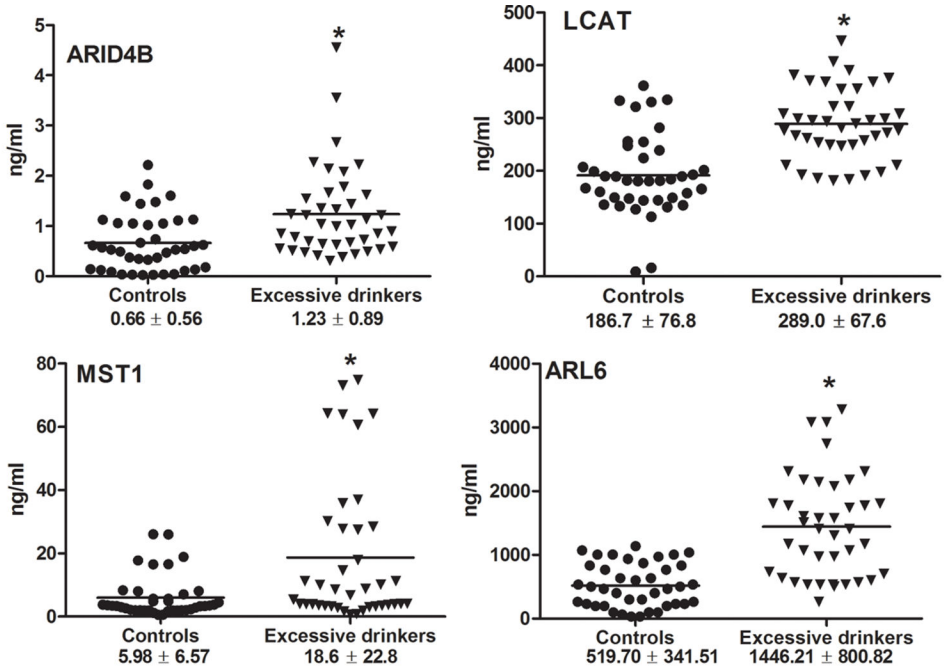


Fig 3.



Protein	Gene Symbol	ELISA fold difference	LC-MS fold difference	ELISA p-value
AT-rich interactive domain-containing protein 4B	ARID4B	1.87	2.5	0.0009
Phosphatidylcholine-sterol acyltransferase	LCAT	1.55	2.1	0.0004
Hepatocyte growth factor-like protein	MST1	3.1	1.9	0.0001
ADP-ribosylation factor 6	ARL6	2.78	1.9	0.0002

Fig 4.

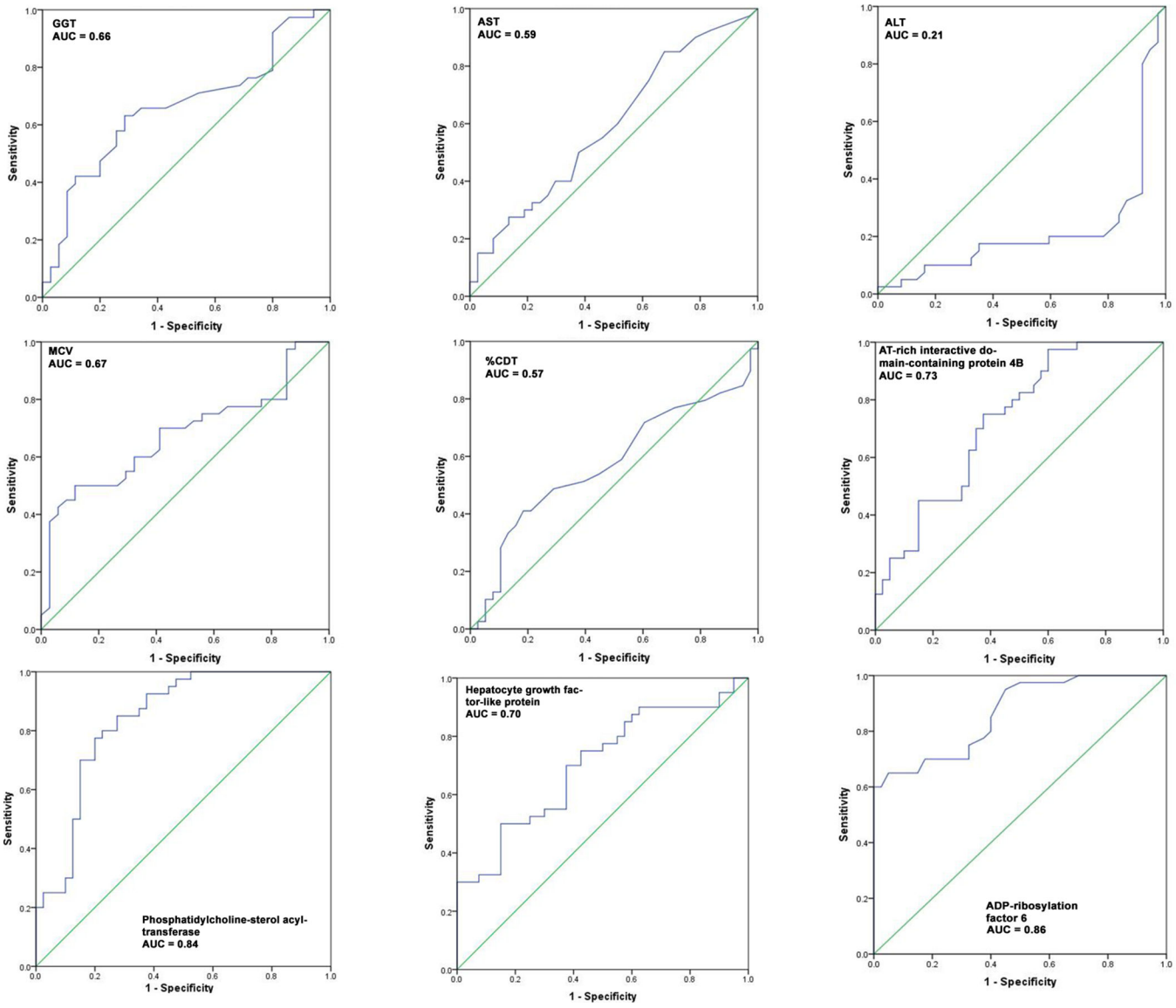


Fig 5.

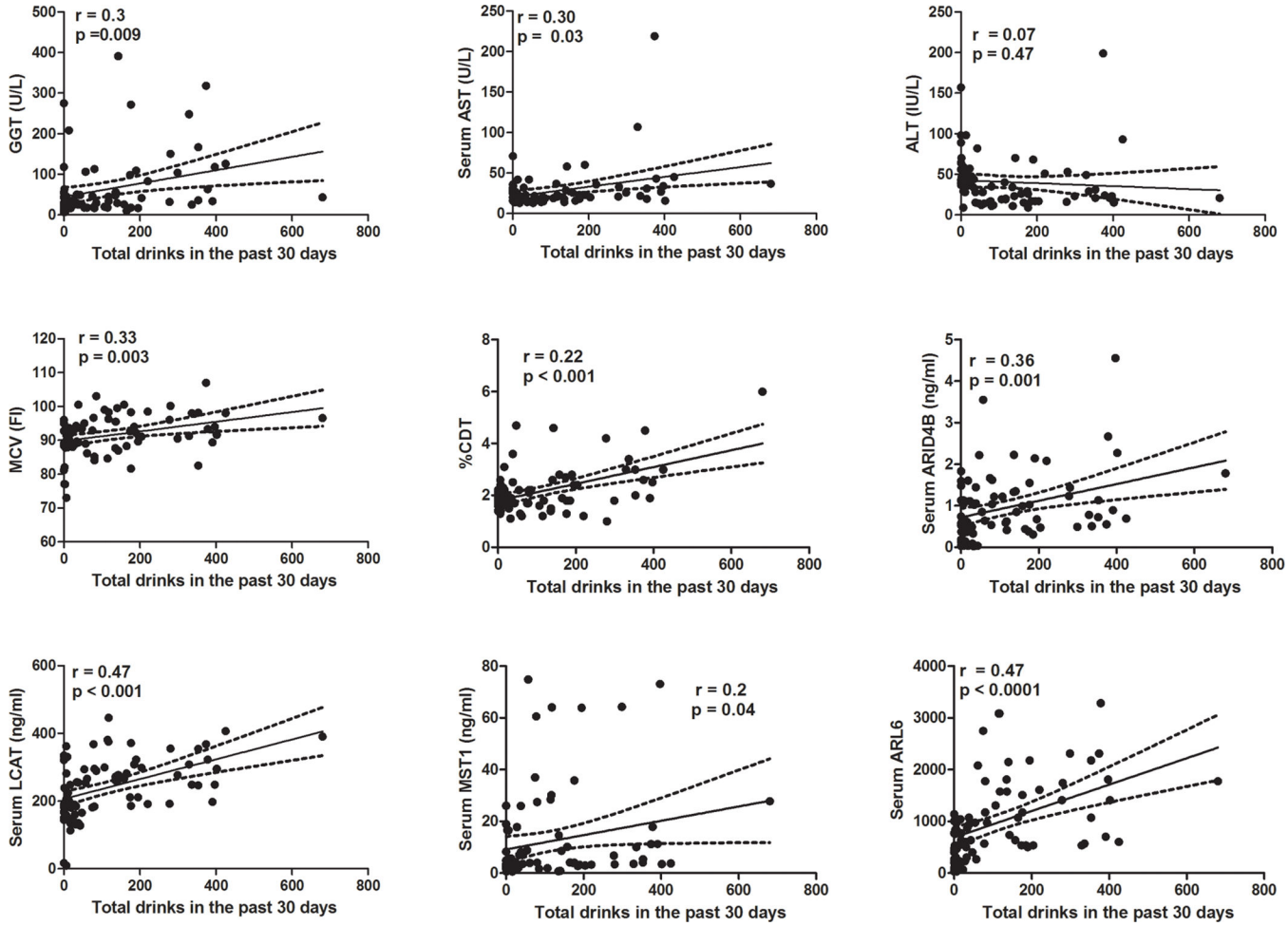


Fig 6.

Table 1

Characteristics of the Discovery and Verificaiton cohorts

Demographic and clinical characterstics	Discovery cohort			Verification cohort		
	Controls (n = 49)	Excessive drinkers (n = 54)	p-value	Controls (n = 40)	Excessive drinkers (n = 40)	p-value
Age (yrs)	39.8 ± 9.2	44.7 ± 11.4	0.10	31.4 ± 4.4	33.2 ± 4.7	0.08
Male sex, n (%)	43 (88%)	41 (76%)	0.12	34 (85%)	33 (82%)	1.00
Race, n (%)						
: White	40 (82%)	45 (83%)	0.32	34 (85%)	35 (87%)	0.80
: Black	4 (8%)	8 (15%)		4 (10%)	4 (10%)	
BMI (kg/m^2)	28.8 ± 4.2	27.9 ± 4.8	0.30	30.1 ± 5.1	27.9 ± 7.1	0.12
AUDIT-C	3.9 ± 3.8	29.2 ± 5.9	0.0001	4.7 ± 5.8	28.7 ± 6.9	0.001
Alcohol drinking patterns during the last 30 days from TLFB						
: Total drinks	14.5 ± 15.3	335.8 ± 141.1	0.0001	13.2 ± 13.8	217.5 ± 137.3	0.0001
: Number of days drinking last 30 days	5.3 ± 4.5	23.8 ± 4.8	0.0001	5.0 ± 5.3	19.3 ± 5.8	0.0001
: Average drinks per drinking day	2.2 ± 2.2	15.2 ± 6.9	0.0001	2.1 ± 2.2	11.0 ± 5.1	0.0001
: Average drinks per day	0.4 ± 0.6	11.1 ± 4.7	0.0001	0.4 ± 0.4	7.3 ± 4.5	0.0001
: Number of heavy drinking days	0	21.4 ± 6.1	0.0001	0	20.3 ± 6.4	0.0001
: Greatest number of drinks in one day	2.8 ± 4.3	19.8 ± 8.4	0.0001	3.6 ± 4.1	17.4 ± 6.9	0.0001
Laboratory measures						
: Serum bilirubin (mg/dL)	0.7 ± 1.3	0.8 ± 0.6	0.40	0.6 ± 0.4	0.6 ± 0.3	0.46
: Serum AST (U/L)	23.5 ± 10.6	31.7 ± 17.1	0.007	23.5 ± 11.1	31.3 ± 16.6	0.02
: Serum ALT (U/L)	32.8 ± 21.0	45.8 ± 19.6	0.002	30.3 ± 10.1	37.6 ± 16.8	0.02
: Serum albumin (g/dL)	4.2 ± 0.4	3.7 ± 0.4	0.001	4.2 ± 0.3	3.6 ± 0.3	0.001
: Serum GGT (U/L)	31.5 ± 16.7	44.9 ± 13.9	0.04	43.6 ± 53.7	82.8 ± 90.0	0.03
: Serum %Carbohydrate deficient transferrin (%CDT, %)	1.4 ± 0.8	2.9 ± 1.4	0.001	1.9 ± 0.6	2.3 ± 1.0	0.009
: Mean Corpusular volume (fL)	89.9 ± 3.9	93.8 ± 6.4	0.001	89.4 ± 5.6	93.3 ± 5.8	0.005

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Table 2

Performance of the new novel proteins in the differential diagnosis of excessive drinkers from controls in comparison to the conventional markers

Test	Cutoff	Sensitivity	Specificity	AUC (95% CI)
GGT	23	0.76	0.71	0.66 (0.53–0.78)
AST	17.5	0.85	0.67	0.59 (0.46–0.72)
ALT	16.5	0.75	0.91	0.21 (0.10–0.32)
MCV	86.5	0.85	0.85	0.67 (0.55–0.80)
%CDT	1.45	0.82	0.86	0.57 (0.44–0.70)
AT-rich interactive domain-containing protein 4B	0.32	0.97	0.70	0.73 (0.62–0.83)
Phosphatidylcholine-sterol acyltransferase	184.1	0.95	0.47	0.84 (0.75–0.93)
Hepatocyte growth factor-like protein	2.07	0.90	0.72	0.70 (0.58–0.81)
ADP-ribosylation factor 6	283.3	0.97	0.65	0.86 (0.78–0.94)

Table 3

The Principle component analysis to determine the ability of each marker or in combination to detect subjects with excessive alcohol use: Proportion of variance explained by the first three principle components

Eigenvalues of the Covariance Matrix			
Principle component	**Eigenvalue**	**Proportion of variance**	**Cumulative proportion of variance**
1	645515.565	0.978	0.978
2	7722.426	0.012	0.990
3	5110.214	0.008	0.998

Table 4

The Principle component analysis to determine the ability of each marker or in combination to detect subjects with excessive alcohol use: Factor loading for the first three components.

Markers	Principal component 1	Principal component 2	Principal component 3
AST	0.005	0.184	0.131
ALT	−0.008	0.156	0.202
MCV	0.002	0.013	−0.004
%CDT	0.000	−0.003	−0.001
GGT	0.011	0.731	0.594
LCAT	0.049	0.637	−0.766
ARID4B	0.000	0.000	0.001
MST1	0.008	0.006	−0.030
ARL6	0.998	−0.039	0.032