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ÁREA DE CONCENTRAÇÃO BIOCIÊNCIAS

THIAGO BELTRAMI DIAS BATISTA

SALIVARY PROTEOME CHARACTERIZATION OF ALCOHOL AND TOBACCO DEPENDENTS

Curitiba
2019

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Doutor em Odontologia, Área de Concentração em Biociências.

Orientador: Profa. Dra. Luciana Reis Azevedo Alanis

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TERMO DE APROVAÇÃO

THIAGO BELTRAMI DIAS BATISTA

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Curitiba, 04 de julho de 2019.

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SUMÁRIO

Title page

Salivary proteome characterization of alcohol and tobacco dependents. *

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Abstract

Background: Alcohol and substances found in tobacco may alter salivary flow and amount of saliva proteins. This study aimed to compare salivary proteins between alcohol dependent smokers and controls. **Methods**: This is a case-control study with men older than 18 years of age, matched by age. The alcohol-dependent group was composed by heavy smokers and alcohol consumers. Unstimulated whole saliva was collected from all subjects. Analysis of digested peptides was performed in mass spectrometer. Data were processed using ProteinLynx GlobalServer software. Results were obtained by searching the *Homo sapiens* database from the UniProt catalog. The search tool IBI-IMIM was used to identify candidate proteins for biomarkers. **Results**: Alcohol-dependent and control groups were composed of nine participants each, with mean age of 36.89±2.57 and 35.78±1.64 years, respectively. 404 salivary proteins were found in both groups; 282 in the alcohol-dependent. Among the 96 proteins presented in both groups, 32 were up-regulated in the alcohol dependents (i.e. “Hemoglobin subunit beta” and “Forkhead box protein P2” were up-regulated at least 10-fold), 23 were down-regulated (i.e. “Statherin” and “RNA-binding protein 25” were down-regulated at least 10-fold), and 41 presented similar expression in both groups. 71 proteins were candidates for biomarkers of disorders; 58 presented in the alcohol dependents’ saliva. The most common disorders were neoplasms, genetic, cardiovascular, metabolic and glandular diseases. **Conclusions**. Salivary protein profile undergoes strong changes in alcohol and tobacco dependents. 34% of salivary proteins present in alcohol and tobacco dependents were present in controls; 14.5% of them were expressed in similar quantity.

Keywords: Proteomics; Saliva; Alcoholism; Tobacco Use; Oral Health

Introduction

Saliva and its components are the main supporter of oral physiology. Its lubricating function facilitates speech, chewing, swallowing, taste, and maintaining dental health and local homeostasis (Cho et al., 2017; Holmberg and Hoffman, 2014). The organic elements of saliva, especially proteins, are constantly secreted, maintaining acquired dental pellicle formation, and soft and hard tissues lubrication (Holmberg and Hoffman, 2014; Proctor and Carpenter, 2014). The concentration of secreted proteins may vary depending on some stimuli. One stimulus pathway is the release of norepinephrine by sympathetic nerve endings binding to β1-adrenoceptors. Signaling from parasympathetic nerves and cholinergic stimuli can also contribute to the release of proteins in saliva (Proctor and Carpenter, 2014).

Chemical substances, such as alcohol and substances found in tobacco, may alter salivary flow and the amount of saliva proteins. They usually alter the salivary constitution indirectly, by systemic changes in the patient's physiology. Alcohol also acts through stimuli in local nerve endings, resulting in decreased salivary flow and local pH changes (Dukić et al., 2013; Enberg et al., 2001; Gelbier and Harris, 1996).

Variations in salivary protein concentration may alter the acquired pellicle formation on enamel surface, resulting in a non-uniform and without multilayer structure with decreased lubrication properties, which may lead to dental demineralization, often present in alcohol users (Zeng et al., 2017). The most common oral conditions are high caries experience, periodontitis and mucosal lesions, besides alcohol being an important risk factor for the development of head and neck carcinomas (Priyanka, 2017; Zygogianni et al., 2011).

To our knowledge, there is no description in the literature of salivary proteomics of alcohol dependent individuals and smokers. Some studies report variations in specific groups of salivary proteins in alcohol dependents, such as proteins related to the immune system (Waszkiewicz et al., 2012, 2008), Epidermal Growth Factor concentration (Benamouzig et al., 1996), TNF-alfa expression (Slomiany et al., 1997), total protein concentration (Enberg et al., 2001), and salivary amylase activity (Enberg et al., 2001).

Describing the salivary protein profile of alcohol dependents associated with smoking may help to understand which mechanisms lead to unfavorable dental and periodontal conditions, and the higher predisposition to benign and malignant oral mucosal lesions. In addition, the salivary protein profile may allow the identification of important systemic biomarkers with current methodologies, using different biological matrices than blood for disease screening. Thus, this study aimed to compare the salivary proteins from alcohol dependent smokers and salivary proteins from non-smokers and non-alcohol dependent users.

Material and Methods

This is a case-control study about the salivary protein composition between alcohol dependent smokers, and non-smokers and non-alcohol dependent individuals matched by age.

This survey was approved by the Local Institutional Review Board under protocol number 1.825.659.

Sample Collection and Processing

Research volunteers were male patients, older than 18 years of age. The alcohol dependent group was composed by individuals recruited from IPTA – Instituto de Pesquisa e Tratamento do Alcoolismo (Campo Largo, Southern Brazil). Inpatients were selected for rehabilitation due to alcohol abuse, who consumed more than 500 mL of ethyl alcohol per week, smokers, with hospitalization time less than 15 days.

The non-alcohol dependent group was composed by patients attended at the Dental Clinic of the Pontifícia Universidade Católica do Paraná (Curitiba, Southern Brazil), matched by age with the alcohol dependent group. All volunteers were informed about the research arrangements and agreed to participate by signing the Free and Informed Consent Form prior to any procedure.

As exclusion criteria, the participants should not have used illicit drugs within 3 months before sample collection, not have systemic diseases, or acute infection symptoms, and not be using antibiotic or anti-inflammatory drugs. For the non-alcohol dependent group, in addition to the exclusion criteria cited above,

smokers and ex-smokers were also excluded, as well as alcohol users who consumed more than 99mL of ethyl alcohol per week.

Nine participants of each group were selected for the present study. The sample size of the groups was based on a previous proteomic study with saliva (Ventura et al., 2018).

All samples were collected between November 2016 and August 2017.

The volunteers were interviewed in relation to socioeconomic data, licit and illicit drugs use profile, systemic health condition, medication use, oral hygiene and xerostomia. According to the tobacco consumption, smokers were classified as mild (less than 10 cigarettes per day), moderate (10 to 19 cigarettes per day), and heavy (more than 20 cigarettes per day) (Albini et al., 2017; Chiolero et al., 2006). After the interview, extraoral clinical examination (palpation of lymph nodes and salivary glands) and intraoral evaluations (inspection and palpation of oral mucosa and inspection of teeth and gingival tissue) were performed, according to the World Health Organization (WHO, 1997). In cases of the presence of oral mucosa changes, dental caries or gingival alterations, the patients were addressed to the Dental Clinic at Pontifical Catholic University of Paraná.

The collection of saliva was performed according to previous research (Ventura et al., 2018). All samples were collected from 9 to 11am. All participants were instructed to perform oral hygiene and stay for 45 min without ingesting any type of food or liquid, smoking cigarettes, or making use of chewable objects. After this time, all patients underwent oral rinsing with 20 mL of distilled water and remained in rest for 15 min. Unstimulated whole saliva was collected in a 50 mL falcon tube immersed in ice. The insoluble components in saliva were separated by centrifugation 3,000 x g for 15 min at 4°C. The supernatant was stored in cryogenic tube at -80°C until samples were processed for proteomic analysis (de Jong et al., 2010; Rhodus et al., 2005; Ventura et al., 2018).

Preparation of the saliva samples

The protocol of proteins extraction was based in a previous study; no IgG depletion columns were used (Ventura et al., 2018). For the extraction, 100 µL of each collected sample was aliquoted in separate tubes. The aliquots were diluted 1:1 with extraction solution, containing 6 M urea, 2 M thiourea and 50 mM NH4HCO3, ph 7.8. After dilution, they were agitated for 10 min at 4°C, then

placed in ultrasonic bath for 5 min and then centrifuged at 14,000 x g at the same temperature for 10 min. This step was repeated twice. After extraction, 3 sample pools were formed. Each pool was composed of 3 samples, chosen by order of collection. The samples were concentrated to 150 µL in Falcon Amicon tubes (Merk Millipore®, Darmstadt, Germany). For reduction of proteins disulfide bridges, 5 mM dithiothreitol (DTT) was used during 40 min at 37°C and subsequently added 10 mM iodoacetamide for 30 min in the dark to prevent cysteine residues from forming new disulfide bonds. The amino acid chains were cleaved with 2% (w/w) trypsin (Promega®, Madison, WI) for 14 hours at 37°C. Then, 10 µL of 5% acid formic were added. The samples were purified and desalted using the C18 Spin columns (Thermo Fisher Scientific®, Waltham, MA) and 1 µL of each sample was used for the protein quantification by the Bradford method (Bio-Rad®, Hercules, CA). The samples were resuspended in the solution containing 3% acetonitrile and 0.1% formic acid and submitted to Nano Liquid Chromatography Electron Spray Ionization Tandem Mass Spectrometry - LC-ESI-MS/MS (Waters, Wilmslow, UK).

Shotgun label-free quantitative proteomic analysis

The analysis of tryptic peptides was performed in the nanoACQUITY UPLC system (Waters, Milford, MA) coupled to Xevo Q-TOF G2 mass spectrometer (Waters, Milford, MA). The nanoACQUITY UPLC system was equipped with a HSS T3 type column (Acquity UPLC HSS T3 column 75 µm x 150 mm; 1,8 µm) (Waters, Milford, MA), previously equilibrated with mobile phase B, 7% (100% Acetonitrile + 0.1% formic acid). Peptides were separated by linear gradient 7 – 85% mobile phase B for 70 min with 0.35 µL/min flow rate, the column temperature was maintained at 45°C. The mass spectrometer was operated in positive ion mode, with 75 min of data acquisition time. Data were processed using ProteinLynx GlobalServer software (PLGS) version 3.03 (Waters, Milford, MA). The identification of saliva proteins was performed using the ion count algorithm incorporated into the software. The results were obtained by searching the Homo sapiens database from the UniProt catalog (Universal Protein Resource) in August 2017 (http://www.uniprot.org). All pool samples were analyzed in triplicates (Consortium, 2017; Ventura et al., 2018).

For label-free quantitative proteome, three MS raw files from each group were analyzed using the Protein Lynx Global Service (PLGS, v 2.2.5, Waters Co., Manchester, UK) software. All the proteins identified with a score with confidence higher than 95% were included in the quantitative statistical analysis embedded in the PLGS software. Identical peptides from each triplicate by sample were grouped based on mass accuracy (<10 ppm) and on time of retention tolerance <0.25 min, using the clustering software embedded in the PLGS. Difference in expression among the groups was calculated using Monte-Carlo algorithm and expressed as $p<0.05$ for proteins present in lower abundance and $1-p>0.95$ for proteins present in higher abundance, when one location was compared to another.

Protein Classification and Bioinformatic analysis

Repeated proteins, fragments and reverse proteins were excluded from the initial data by manual review. The salivary proteins presented in each group (alcohol dependent group and non-alcohol dependent group) were compared and classified according to expression and later classified as up-regulated in the tested group (alcohol dependent group), similar expression in both groups or down-regulated in the tested group.

Proteins were classified by molecular function, cellular component, and biological process involved, according to the terms described by the Princeton University generic gene term mapper (Generic Gene Ontology - GO - Term Finder). The search was performed using the GOA database for Homo sapiens (Boyle et al. 2004; Princeton University). The search tool IBI-IMIM was used to identify candidate proteins for biomarkers (Database of disease-related biomarkers) (Bravo et al., 2014; Cho et al., 2017).

Results

Salivary protein profile

Alcohol-dependent and non-alcohol dependent groups were composed of nine participants each, all males, with mean age of 36.89 ± 2.57 and 35.78 ± 1.64 years, respectively. The mean alcohol use time in the first group was 14.89 ± 9.25 years and all participants were heavy smokers with consumption greater than 20

cigarettes per day. The mean smoking time was 18.89 ± 8.54 years until the time of collection.

The amount of total protein in the triplicates after Bradford analysis were 37.11 µg, 40.43 µg and 52.30 µg in the alcohol dependent group and 33.28 µg, 49.62 µg and 62.00 µg in the non-alcohol dependent group. In total, 404 proteins were found in the saliva from both groups, with 282 presented in the alcohol-dependent group. The way the proteins were distributed in each group can be visualized in Fig. 1.

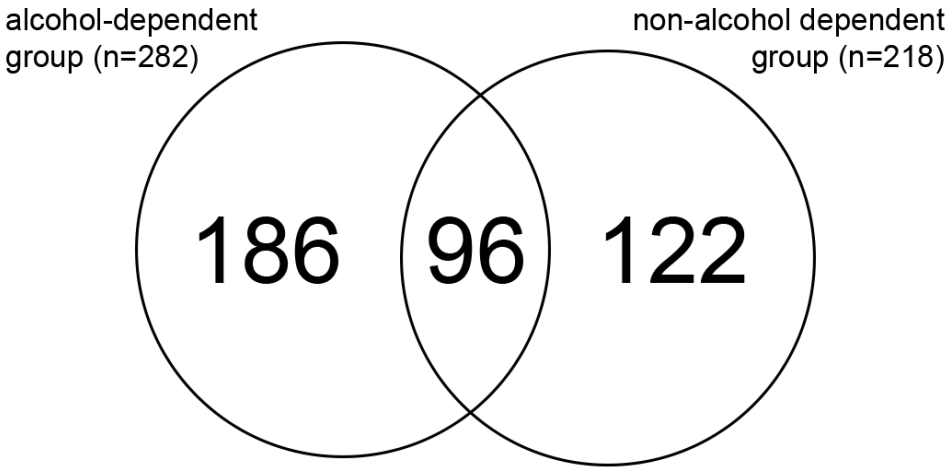

Fig. 1. Venn diagram with the number of proteins detected in saliva of each group.

According to the protein salivary concentration (difference in expression), among the 96 proteins presented in both groups, 32 were up-regulated in the alcohol dependent group, 23 were down-regulated, and 41 proteins presented similar expression in both groups according to the Monte Carlo test. In the saliva of alcohol and tobacco dependents, “Hemoglobin subunit beta” and “Forkhead box protein P2” were the two most up-regulated proteins compared to the control group, with ratios of 17.81 and 10.07, respectively, and “Statherin” and “RNA-binding protein 25” were the two most down-regulated salivary proteins, with ratios of 0.03 and 0.10, respectively (Table 1).

All identified proteins, including their differences in expression, biological processes, molecular functions and cellular components are described in the supplementary material. The amount of proteins (GO) according to their molecular function, cellular component and biological processes are presented in Fig. 2.

Table 1
Proteins presents in saliva of both groups and its expression differences.

Expression differences	Ratio A/C	Score	Accession Number	Protein name
↑	1.93	1740.62	P68032	Actin alpha cardiac muscle 1
↑	2.12	1753.68	P68133	Actin alpha skeletal muscle
↑	2.16	1740.62	P62736	Actin aortic smooth muscle
↑	2.29	4796.52	P60709	Actin cytoplasmic 1
↑	2.14	4796.52	P63261	Actin cytoplasmic 2
↑	2.46	1740.62	P63267	Actin gamma-enteric smooth muscle
↑	1.34	410.33	P04280	Basic salivary proline-rich protein 1
↑	1.35	4894.05	P01036	Cystatin-S
↑	1.03	3222.1	P01037	Cystatin-SN
↑	17.81	589.65	O15409	Forkhead box protein P2
↑	1.79	238.64	Q86W71	GOLGA4 protein
↑	1.75	268.95	Q13439	Golgin subfamily A member 4
↑	7.61	5534.44	G3V1N2	HCG1745306_ isoform CRA_a
↑	5.05	8580.57	P69905	Hemoglobin subunit alpha
↑	10.07	6003.34	P68871	Hemoglobin subunit beta
↑	4.06	1171.27	P02042	Hemoglobin subunit delta
↑	5.26	387.65	P69891	Hemoglobin subunit gamma-1
↑	4.85	387.65	P69892	Hemoglobin subunit gamma-2
↑	1.52	521.61	O60479	Homeobox protein DLX-3
↑	1.35	432.62	Q86Y46	Keratin_ type II cytoskeletal 73
↑	3.03	443.41	Q6GTX8	Leukocyte-associated immunoglobulin-like receptor 1
↑	2.77	382.08	P31025	Lipocalin-1
↑	1.73	1050.7	P61626	Lysozyme C
↑	2.97	1170.4	Q6S8J3	POTE ankyrin domain family member E
↑	2.89	1170.4	A5A3E0	POTE ankyrin domain family member F
↑	5.53	316.29	Q8IZS8	Voltage-dependent calcium channel subunit alpha-2/delta-3

↑	1.03	1324.57	P09228	Cystatin-SA
↑	4.53	207.1	Q6IQ26	DENN domain-containing protein 5A
↑	1.15	986.28	P12273	Prolactin-inducible protein
↑	2.23	310.65	A6NNI4	Tetraspanin
↑	1.01	27231.79	P04745	Alpha-amylase 1
SE	2.23	305.51	Q12955	Ankyrin-3
SE	1.02	3074.69	P02768	Serum albumin
SE	1.43	485.16	Q75MZ5	Forkhead box P2_ isoform CRA_a
SE	1.04	494.51	Q0PRL4	Forkhead box P2 variant 3
SE	1.03	128.88	Q96QU1	Protocadherin-15
SE	1.12	506.33	O60315	Zinc finger E-box-binding homeobox 2
SE	1.03	411.65	Q9NP78	ATP-binding cassette sub-family B member 9
SE	1.03	255.08	Q9UKM9	RNA-binding protein Raly
SE	1.03	1036.56	P01721	Immunoglobulin lambda variable 6-57
SE	1.04	160.92	Q9BZX4	Ropporin-1B
SE	1.04	274.65	P05161	Ubiquitin-like protein ISG15
SE	1.06	190.4	Q15436	Protein transport protein Sec23A
SE	1.84	387.65	P02100	Hemoglobin subunit epsilon
SE	1.02	333.65	O15021	Microtubule-associated serine/threonine-protein kinase 4
SE	1.08	255.56	Q14232	Translation initiation factor eIF-2B subunit alpha
SE	1.04	202.78	Q96IY1	Kinetochore-associated protein NSL1 homolog
SE	0.97	193.41	A6NDL8	Olfactory receptor 6C68
SE	1.04	237.38	Q15283	Ras GTPase-activating protein 2
SE	1.02	302.1	O14795	Protein unc-13 homolog B
SE	1.03	518.87	Q6ZSG1	E3 ubiquitin-protein ligase RNF165
SE	1.15	196.81	Q5THK1	Protein PRR14L
SE	1.04	278.7	Q8N9H6	Uncharacterized protein C8orf31

SE	1.03	197.98	O00231	26S proteasome non-ATPase regulatory subunit 11
SE	1.03	348.26	Q15582	Transforming growth factor-beta-induced protein ig-h3
SE	1.06	250.34	Q86XF7	Zinc finger protein 575
SE	1.02	284.48	O95780	Zinc finger protein 682
SE	1.02	330.32	Q8WXB4	Zinc finger protein 606
SE	1.03	297.54	B4E159	Zinc finger protein 721
SE	1.01	260.6	Q14002	Carcinoembryonic antigen-related cell adhesion molecule 7
SE	0.99	260.56	A6NEH6	Transmembrane protein 247
SE	0.98	316.62	Q765P7	MTSS1-like protein
SE	1.00	345.48	H0YIY4	Gephyrin (Fragment)
SE	0.98	365.38	P01034	Cystatin-C
SE	1.01	226.42	O15375	Monocarboxylate transporter 6
SE	1.00	301.73	Q5VSP4	Putative lipocalin 1-like protein 1
SE	0.94	525.96	Q96DA0	Zymogen granule protein 16 homolog B
SE	0.99	19911.77	P19961	Alpha-amylase 2B
SE	0.84	302.47	Q6NXT4	Zinc transporter 6
SE	0.84	330.69	Q9BZF1	Oxysterol-binding protein-related protein 8
SE	0.95	763.32	P13646	Keratin type I cytoskeletal 13
SE	0.83	1158.14	Q9P2R6	Arginine-glutamic acid dipeptide repeats protein
SE	0.76	1062.35	P0CG38	POTE ankyrin domain family member I
↓	0.61	545.37	P18615	Negative elongation factor E
↓	0.55	181.39	P0CG39	POTE ankyrin domain family member J
↓	0.87	2956.72	P02812	Basic salivary proline-rich protein 2
↓	0.75	736.45	Q562R1	Beta-actin-like protein 2
↓	0.47	240.22	Q9NQY0	Bridging integrator 3

↓	0.40	172.46	P23280	Carbonic anhydrase 6
↓	0.40	1242.75	P15515	Histatin-1
↓	0.36	1349.11	P15516	Histatin-3
↓	0.70	2608.5	P01876	Immunoglobulin heavy constant alpha 1
↓	0.70	2252.35	P01877	Immunoglobulin heavy constant alpha 2
↓	0.54	1650.72	P01591	Immunoglobulin J chain
↓	0.31	349.5	P20592	Interferon-induced GTP-binding protein Mx2
↓	0.87	675.64	P19013	Keratin type II cytoskeletal 4
↓	0.40	688.79	Q9NYZ2	Mitoferrin-1
↓	0.95	18247.75	P04746	Pancreatic alpha-amylase
↓	0.72	592.56	P01833	Polymeric immunoglobulin receptor
↓	0.14	265.2	Q5W0V3	Protein FAM160B1
↓	0.10	328.02	P49756	RNA-binding protein 25
↓	0.28	3979.31	P02810	Salivary acidic proline-rich phosphoprotein 1/2
↓	0.22	190.19	H0YDR5	Single Ig IL-1-related receptor (Fragment)
↓	0.59	251.96	Q8WXA9	Splicing regulatory glutamine/lysine-rich protein 1
↓	0.03	1767.57	P02808	Statherin
↓	0.76	6879.29	P02814	Submaxillary gland androgen-regulated protein 3B
↓	0.15	579.79	Q8TAF7	Zinc finger protein 461

Note: SE = similar expression compared to control group; ↑ = up-regulated (1-p>0.95); ↓ = down-regulated (p<0.05); Ratio A/C = ratio between alcoholic-dependent and control group proteins.

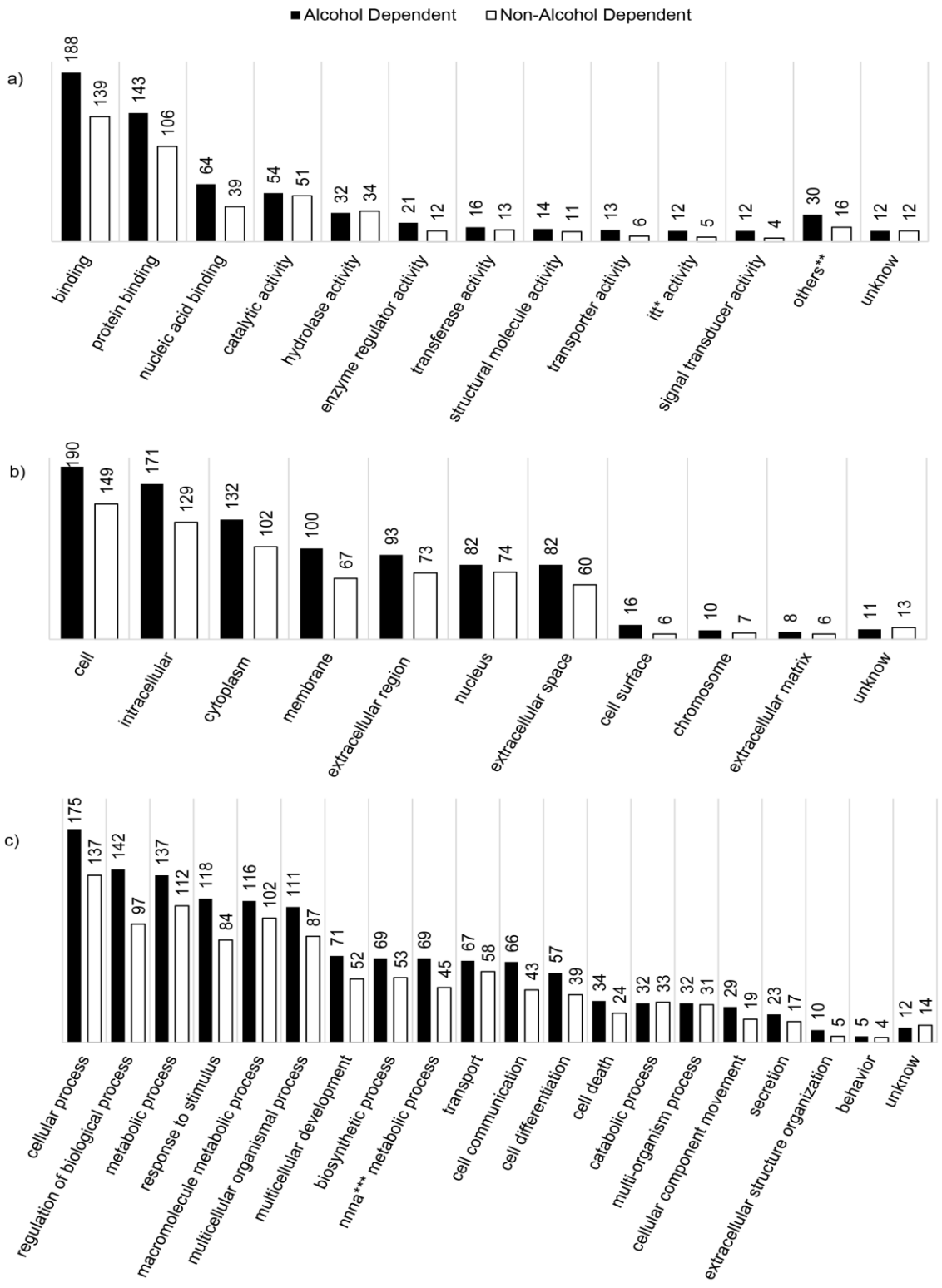

Fig. 2. Number of proteins divided by group and classified by molecular function (a), cellular component (b) and biological processes involved (c).
Note: *itt = ion transmembrane transporter; **others = kinase activity, oxidoreductase activity, channel activity, lyase activity, helicase activity, ligase activity, antioxidant activity, translation regulator activity, electron carrier activity, protein transporter activity, isomerase activity; ***nnna = nucleobase, nucleoside, nucleotide and nucleic acid.

Salivary biomarkers

The salivary proteins candidates for biomarkers found with IBI-IMIM search tool were divided according to presence and absence in the saliva of each group and by difference in expression (Table 2). Among 404 salivary proteins detected by proteomic analysis, 71 were candidates for biomarkers of local and systemic alterations, with 58 (81.7%) of them presented in the saliva from alcohol dependents individuals.

The most common disorders to which salivary proteins were indicated as candidates for biomarkers are as follows: neoplasms (n= 47), genetic diseases (n= 44), cardiovascular diseases (n= 36), metabolic and glandular diseases (n=36), hepatobiliary and pancreatic diseases (n= 35) (Fig. 3).

Table 2
Proteins candidates for biomarkers per group and divided by expression difference.

Salivary Protein Expression	Proteins candidate for biomarkers n (%)	Total proteins n
Only in Alcohol Dependents group	32 (17.2)	186
Only in Non-Alcohol Dependents group	13 (10.6)	122
Similar expression in both groups	8 (19.5)	41
Up-regulated proteins in Alcohol Dependents group in relation to Non-Alcohol Dependents group	14 (43.7)	32
Down-regulated proteins in Alcohol Dependents group in relation to Non-Alcohol Dependents group	4 (17.4)	23

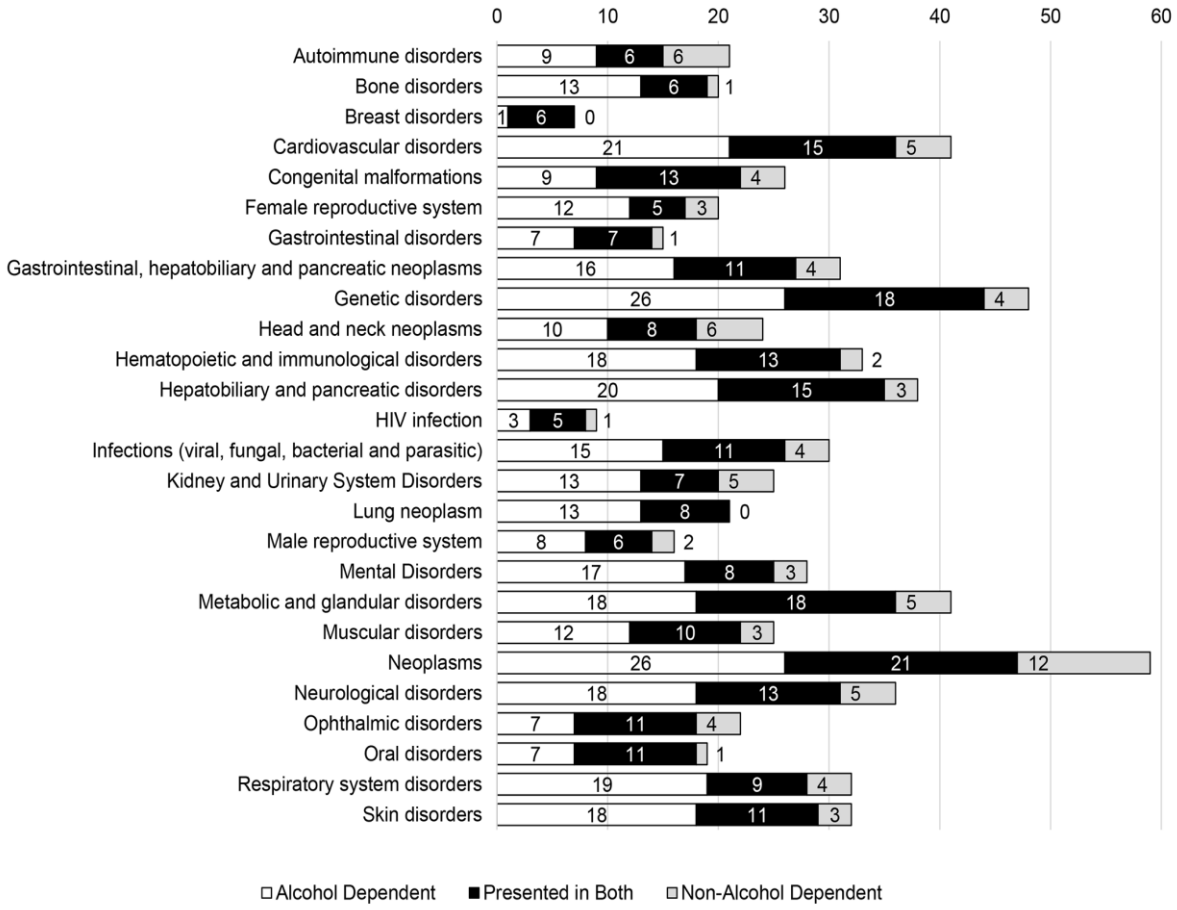

Fig. 3. List of proteins candidate for biomarkers, classified by groups of diseases presented by group.

Discussion

Chronic and acute alcohol consumption may lead to different changes in body physiology. The amount and variability of proteins presented in saliva may be altered by alcohol abuse, either by local stimuli or systemic physiological changes (Proctor and Carpenter, 2014). Alcohol is responsible for decreasing stimulated and non-stimulated salivary flow (Enberg et al., 2001). The difference of salivary protein composition presented in this study reflects the different habits between alcohol dependent and non-alcohol dependent groups. Among 404 salivary proteins identified in the two groups, only 96 were present in both of them, of which 41 did not show differences in expression between groups. In addition, the number of salivary proteins candidates for biomarkers from the alcohol-dependent group was higher compared to the salivary proteins from the non-alcohol dependents, especially for neoplastic, cardiovascular, hepatobiliary

and pancreatic diseases. However, it is valid to emphasize that the results of this study should be interpreted as changes caused by longtime exposure to alcohol without the acute effects at the time of the analysis.

In this study, proteins presented in both groups represent those typically found in saliva, such as actin, albumin, amylases, hemoglobin chains, immunoglobulin chains, cystatins, histatins, lysozyme, keratins, salivary proline-rich proteins, and statherin. Although the presence of keratin may denote normal differentiation of oral epithelial tissue, some types of keratin may result from contamination by contact between skin and saliva at the time of collection (Baliban et al., 2012; Ventura et al., 2018). The keratins identified in this study were one of type I (keratin 13) and two of type II (keratin 4 and 73). Keratin 13 and 73 are generally found in skin glands and hair follicles, although keratin 13 can also be found in esophageal epithelial and type 4 in the oral mucosa (Consortium, 2017). As mentioned previously, this may occur during the collection procedure, especially in debilitated patients, such as in the present sample.

Molecules involved in the immune response were found with altered concentrations in the saliva from alcohol dependents of the present study. Changes in the immune system of these individuals have been described and reported in other studies (Curtis et al., 2013). Thereafter, exposure to alcohol compromises the immune system action against infections, resulting in longer recovery times, longer hospitalization time, and higher morbidity and mortality numbers when compared to non-intoxicated individuals (Curtis et al., 2013; Messingham et al., 2002). In this study, among the main proteins involved with the immune system with lower concentrations in alcohol dependents saliva are the various immunoglobulin chains (apart from Immunoglobulin lambda variable 6-57) and histatins 1 and 3. Conversely, there was an increase of C-lysozyme in the alcohol dependents saliva, which can be explained as an innate immune system compensation, caused by increased phagocytosis by local macrophages, and the non-detection of mucin-7 in the saliva of this group, which show bacterial clearance function in the mouth (Consortium 2017).

The proteins presented in higher concentration in the alcohol dependents saliva varied, highlighting actin, cystatine, hemoglobin and alpha-amylase. Actin are intracellular cytoplasmic filaments which are part of the composition of the cytoskeleton. They are proteins associated with motility and support the cellular

structure. They were also identified as possible biomarkers for the differentiation between premalignant and malignant lesions in the oral mucosa (de Jong et al., 2010). This increased concentration may be associated with alcohol-dependent group habits, since alcohol and tobacco are substances known to be carcinogenic. One type of malignant disease caused by alcohol and tobacco use is oral cancer (Secretan et al., 2009). Further studies are needed to confirm salivary concentrations of actin associated with differentiation between premalignant and malignant lesions in oral mucosa, as inflammatory processes that causes tissue necrosis can release actin filaments in saliva (Blotnick et al., 2017; Geijtenbeek, 2012).

Hemoglobin is a blood protein responsible for oxygen transport to various tissues. The increased presence in alcohol-dependents saliva may be associated with tissue injury and blood hemolysis. In addition, chronic alcohol users may develop macrocytic anemia due to lack of nutrients, especially folate. The ethanol concentrations in bloodstream may destabilize the erythrocyte membrane, leading to intravascular hemolysis (Andersen et al., 2012; Tyulina et al., 2000). Increased salivary hemoglobin may be harmful to oral mucosa, considering its high potential to cause damage to mucosa, which occurs due to the presence of heme group, which is quite reactive. In intravascular space, hemoglobin causes endothelial dysfunction, vascular damage and inflammation (Schaer et al., 2013).

Still involving the participation of red blood cells and related enzymes, mitoferrin 1 appears in low concentration in saliva of alcohol dependents in this study. It is an enzyme responsible for transporting iron through the mitochondrial membranes of erythroblasts. It is usually expressed in large quantities in hematopoietic tissues, and its deficiency makes it difficult to incorporate iron into the heme group, which may result in hypochromic anemia and differentiation delay of immature erythroblasts (Ye and Rouault, 2010).

Proteins that strongly adhere to the enamel pellicle are mucins, statherins, and proline-rich proteins. Acute alcohol consumption causes deformations in film composition (Zeng et al., 2017). In the present study, decreased statherins concentration was detected in alcohol dependents saliva with chronic use. In addition to statherins, several proline-rich proteins were present in decreased concentration or were absent; only 1 (basic salivary proline-rich protein 1) was present in higher concentration in the alcohol-dependents saliva, and mucin 7 was

only found in the saliva of non-alcohol dependents (Douglas et al., 1991; Elangovan et al., 2007; Tabak, 1995; Zeng et al., 2017). Decreased statherin is also associated with the presence of dental calculus, possibly due to alterations related to acquired pellicle (Pateel et al., 2017).

Carbonic anhydrase is an enzyme that controls the pH by reversibly catalyzing hydration of carbon dioxide. The type 6 enzyme, described in the supplementary material, is the enzyme commonly found in saliva. It was found with decreased concentrations in the alcohol-dependent group, which may result in pH salivary changes even after alcohol use. It also seems to be involved in taste (Hassan et al., 2013; Lindskog, 1997).

In the present study, the number of proteins candidates for biomarkers in the alcohol dependent group was high, mainly in neoplastic, cardiovascular, hepatobiliary and pancreatic diseases. The fact that the group tested was composed of alcohol-dependent smokers may have contributed to an increased number of salivary biomarkers, since, according to some epidemiological studies (Corrao, 2004; Fagerström, 2002), the number of systemic changes in this population is higher than in healthy individuals. The main diseases related to alcohol abuse are neoplastic lesions in mouth, pharynx, esophagus, larynx, colon, rectum, liver, and breast. The non-neoplastic diseases are hypertension, coronary disease, ischemic and hemorrhagic stroke, gastroduodenal ulcer, liver cirrhosis and chronic pancreatitis, as well as health injury caused by accidents and violence (Corrao, 2004). Neoplastic lesions for smokers are also common, such as neoplastic disease of the esophagus, lung, bladder, kidney, stomach and pancreas. As well as non-neoplastic diseases, such as chronic obstructive pulmonary disease, coronary disease, stroke, peripheral vascular disease and ulcer (Fagerström, 2002). Despite this, it is important to emphasize that the presence of salivary biomarkers does not indicate the presence of disease, since there are several biomarkers that are proteins found in healthy patients. It will only direct the diagnosis of disease when proteins present changes, for example, high or low concentration, or chain mutations which lead to conformational changes in protein structure (FDA-NIH Biomarker Working Group, 2016). Describing these proteins and their differences in expression between groups is extremely important to serve as basis for new researches that focuses on biological detection and biomarkers description of some diseases.

Biomarkers are biological molecules used as indicators of normal or pathogenic biological processes or monitoring physiological events on drugs action. There are several types, such as diagnostic, monitoring and response biomarkers, among others (FDA-NIH Biomarker Working Group, 2016; Ghallab, 2018). The search tool of IBI-IMIM group was elaborated from a research data collection tool. There is vast amount of information on biomarkers related to physiological and pathological changes in scientific literature; such tool provides gene identification related to possible biomarkers by means of MeSH terms data mining in MEDLINE publications (Bravo et al., 2014). However, it is important to point out weaknesses in this tool, since it is an automatic mining tool that uses specific algorithms. It does not guarantee that all proteins candidates for biomarkers reported in the scientific research are present in these data, neither all listed proteins will prove to be biomarkers; for example, some studies refer biomarkers to the appearance of secondary diseases not directly related to primary disease (data not shown). Biomarkers pointed by this tool must be manually reviewed before confirmation.

The fact that no female population was assessed is one limitation of the present study. However, based on the fact that the inclusion of female patients could present changes in salivary protein secretion due to hormonal variations (Abrao et al., 2014; Rukmini et al., 2018) and that only male patients over 18 years of age are attended at IPTA, we assumed the inclusion of only male subjects in the present study. Besides this, the individual health condition was obtained by self-report, therefore with possible bias. To reduce the risk of bias, questions regarding the continuous use of medications were also performed, which helped to clarify possible unreported diseases. Meanwhile, we believe that hepatic, renal and cardiovascular damage in discrete degrees may have been present in our sample but are representative of chronic alcohol and tobacco users. Finally, the alcohol-dependent group was composed by heavy smokers, which make it unfeasible to know which proteins were changed due to the alcohol and which due to the smoking habit. In a pilot study, we observed that most of the alcohol-dependent volunteers were smokers classified as heavy users of tobacco. Thus, we decided to assume that tobacco consumption is a characteristic of alcohol dependent population due to its high frequency and amount, and that the

changes caused by the synergism of both drugs may be even more important than the changes caused by only one.

Proteomic analysis is a powerful tool for protein description of complex biological matrices such as saliva. Therefore, standardization is required in preanalytical phase (Kwasnik et al., 2016; Ventura et al., 2018). Age-paired groups was an option to reduce bias, as well as the option to perform the study only with male volunteers. Conversely, the protein variety between both groups may represent just part of the changes caused by chronic alcohol consumption. Less evident is the difference demonstrated by ethnic variability present in the population, or daily habits, either hygiene habits and other factors that may interact in individual's physiology. Therefore, protein differences found in each group may be a limitation presented by this methodology, in the detection of proteins in low concentrations. The use of pools with small numbers of patients in proteomic studies can reduce such variations, avoiding the dilution of proteins present in few individuals (Ventura et al., 2018; Winck et al., 2015).

With more robust studies involving proteomic analysis associated with decreased material costs, this analysis may become more frequent in clinical testing laboratories.

Conclusion

The salivary protein constitution undergoes strong changes in alcohol and tobacco dependents. The applied methodology allowed identification of 404 proteins in both alcohol dependent and non-alcohol dependent groups. Only 34% of proteins present in the saliva of alcohol-dependent smokers were present in the saliva of non-dependents, and 14.5% of them were expressed in similar quantity. In the saliva of alcohol and tobacco dependents, "Hemoglobin subunit beta" and "Forkhead box protein P2" were up-regulated at least 10-fold, and "Statherin" and "RNA-binding protein 25" were down-regulated at least 10-fold compared to controls. In addition, the number of candidates for biomarkers in the saliva of alcohol-dependent smokers was higher than that on the saliva of non-alcohol dependents, with a high number of biomarkers for disorders compatible with this behavior, especially neoplasms, cardiovascular disorders, hepatobiliary and pancreatic disorders.

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Conflict of interest

No conflicts were declared.

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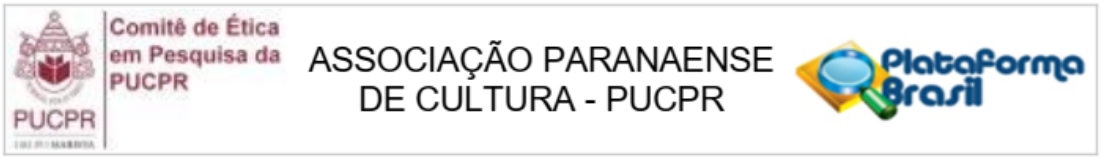
ANEXOS

Parecer do comitê de ética



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Saúde bucal em usuários de crack.
Pesquisador: Luciana Reis Azevedo Alanis
Área Temática:
Versão: 2
CAAE: 60172316.5.0000.0020
Instituição Proponente: Pontifícia Universidade Católica do Parana - PUCPR
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.825.659

Apresentação do Projeto:
Considerado um problema de saúde pública mundial, a cocaína e o crack atingem mundialmente uma faixa de 17 a 20 milhões de pessoas, sendo que os maiores consumidores são América do Norte, América do Sul, Europa Central e Europa Ocidental. Somente nos Estados Unidos, no ano de 2010, os gastos para tratamento/reabilitação dos usuários foram próximos a 28 bilhões de dólares. No Brasil, entre as substâncias ilícitas, a cocaína e o crack são, respectivamente, a segunda e a terceira droga com maior número de usuários adultos. Foram relatados aproximadamente 1 milhão
de usuários de crack em 2012, excluindo-se moradores de rua, segundo o último levantamento realizado no país. Um ponto importante a ser levantado é que usuários de cocaína podem desenvolver imunossupressão como consequência da utilização da droga, uma vez que são drogas imunossupressoras que agem em ampla gama de leucócitos modulando a liberação de citosinas em células natural killer, neutrófilos e macrófagos. Além disso, usuários de cocaína possuem alta prevalência de anorexia e má nutrição, que podem levar ao surgimento de lesões na mucosa bucal. Glossodinia, queilite angular e outras formas de candidose estão entre as mais comuns. A Candida é o gênero responsável pela infecção fúngica mais comum na boca e, somente em casos específicos, o fungo irá se tornar patogênico, como por exemplo, quando ocorrer imunossupressão no indivíduo. No entanto, apesar das drogas apresentarem ação

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imunossupressora, a frequência de infecções fúngicas bucais é muito baixa nos usuários. Como algumas proteínas têm ação preventiva no desenvolvimento da candidose em outras doenças, entre elas as imunoglobulinas e a histatina salivar 5, que possuem ação fungicida, uma forma de elucidar esse comportamento é a realização da análise proteômica da saliva desses indivíduos, além de ser uma estratégia que identifica possíveis biomarcadores para doenças bucais e sistêmicas. Com o aumento do número de dependentes de crack no Brasil, apontado por levantamentos recentes, é comum que profissionais da saúde, principalmente cirurgiões-dentistas, se deparem com usuários em seus atendimentos, fazendo necessário o esclarecimento dos seus efeitos na boca, identificando alterações causadas pela droga sobre a fisiologia local. Estudos para analisar o comportamento da Candida spp. após a exposição fumada em usuários crônicos de cocaína/crack, estudos in vitro e avaliações sialoquímica, sialométrica e das alterações nos fatores de virulência expressas pelo fungo exposto a
droga ajudarão a elucidar a relação crack/candidose. O objetivo do presente estudo é avaliar os efeitos da cocaína na forma de crack sobre modificações na expressão de fatores de virulência da Candida spp, alterações na concentração de componentes da saliva, além de avaliar correlações entre patógeno e hospedeiro usuário de crack.

Objetivo da Pesquisa:

Objetivo Primário:

O objetivo do presente estudo é avaliar os efeitos da cocaína na forma de crack sobre modificações na expressão de fatores de virulência da Candida spp, alterações na concentração de componentes da saliva, além de avaliar correlações entre patógeno e hospedeiro usuário de crack.

Objetivo Secundário:

Nos usuários de crack e álcool: - Avaliar a prevalência de cárie dental pelo índice CPOD (cariado, perdido, obturado); - Avaliar a prevalência de doença periodontal pelos Índices de Placa e Gengival; - Avaliar a presença da xerostomia;- Avaliar a ocorrência de manifestações na mucosa bucal; - Avaliar a sialometria (fluxo salivar estimulado); - Avaliar a sialoquímica: pH, capacidade tampão da saliva, concentração de uréia, glicose, cálcio, proteínas totais e atividade da -amilase; - Avaliar os níveis salivares de histatina-5; - Realizar análise proteômica da saliva. Nos ensaios in vitro: - Avaliar fatores de virulência de Candida albicans. Nos ensaios em modelo animal: - Avaliar volume de perda óssea em ratas submetidas a doença periodontal induzida por ligadura.

Avaliação dos Riscos e Benefícios:

Riscos e Benefícios atendem ao disposto nas resoluções da CONEP.

Comentários e Considerações sobre a Pesquisa:

A pesquisa atende ao disposto nas resoluções da CONEP.

Considerações sobre os Termos de apresentação obrigatória:

Os Termos de apresentação obrigatória atendem ao disposto nas resoluções da CONEP.

Recomendações:

As correções solicitadas foram efetuadas não são necessárias novas recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Projeto aprovado.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_776583.pdf	03/11/2016 20:47:54		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Thiago.docx	03/11/2016 20:47:09	Luciana Reis Azevedo Alanis	Aceito
Declaração de Instituição e Infraestrutura	Autorizacao_san_julian.pdf	18/10/2016 14:02:18	Luciana Reis Azevedo Alanis	Aceito
Outros	Ficha_questionario_saude.pdf	18/10/2016 13:22:21	Luciana Reis Azevedo Alanis	Aceito
Declaração de Pesquisadores	Termos_Thiago.doc	17/09/2016 00:41:04	Luciana Reis Azevedo Alanis	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Thiago.doc	17/09/2016 00:23:53	Luciana Reis Azevedo Alanis	Aceito
Folha de Rosto	Folha_de_rosto_Thiago.docx	17/09/2016 00:23:27	Luciana Reis Azevedo Alanis	Aceito
Cronograma	Cronograma_Thiago.docx	17/09/2016 00:16:48	Luciana Reis Azevedo Alanis	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Continuação do Parecer: 1.825.659

CURITIBA, 18 de Novembro de 2016

Assinado por:
NAIM AKEL FILHO
(Coordenador)

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TCLE - Termo de consentimento livre e esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Eu, _____ (nome completo), RG _____ ,

_____ (nacionalidade), _____ (idade), _____ (estado civil),

_____ (endereço), _____ (profissão),

estou sendo convidado a participar de um estudo denominado "Saúde Bucal Em Usuários De Crack" cujos objetivo é verificar se existe algum tipo de alteração nos componentes da saliva de usuários de *crack*, usuários de cigarro e bebida alcoólica e não usuários de drogas.

A minha participação no referido estudo será no sentido de doação de uma amostra de saliva e células, a saliva será obtida por mim pela deposição dela em um tubo fornecido pelo pesquisador durante 5 minutos. Ele também irá coletar as células da minha bochecha (mucosa jugal) com uma leve raspagem utilizando uma espátula de madeira esterilizada e uma pequena escova de cerdas macias no lado da língua**.** Também fui esclarecido que serei entrevistado com questões referentes a minha condição social, econômica e de saúde, incluindo sobre uso de drogas. Além disso receberei avaliação bucal realizada por profissional qualificado.

Fui alertado de que, da pesquisa a se realizar, posso esperar alguns benefícios, tais como:

1. Orientações sobre higiene oral (ensino da técnica de escovação e uso do fio dental).

2. Encaminhamento e realização de tratamento odontológico na clínica da PUCPR.

Recebi, por outro lado, os esclarecimentos necessários sobre os possíveis desconfortos e riscos decorrentes do estudo, levando-se em conta que é uma pesquisa, e os resultados positivos ou negativos somente serão obtidos após a sua realização. Assim, por se tratar de um procedimento não invasivo, não sofrerei nenhum tipo de ferimento, picada, choque ou qualquer sensação de dor, somente um leve desconforto ou leve coceira na coleta. Estou ciente de que minha privacidade será respeitada, ou seja, meu nome ou qualquer outro dado ou elemento que possa, de qualquer forma, me identificar, será mantido em sigilo. Também fui informado de que posso me recusar a participar do estudo, ou retirar meu consentimento a qualquer momento, sem precisar justificar, e de, por desejar sair da pesquisa, não sofrerei qualquer prejuízo à assistência que venho recebendo.

Os pesquisadores envolvidos com o referido projeto são: **Cassiano Chaiben**, **Indiara Welter Henn**, **Milena Binhame Albini** e **Thiago Beltrami Dias Batista**, alunos do programa de pós graduação em odontologia na PUCPR que poderão ser contatados pelos e-mails: "mibalbini@gmail.com", "tbdbatista@gmail.com" e telefones 3501- 8494, 3010-2563, realizando a pesquisa sob orientação da profa. **Dra. Luciana Reis Azevedo Alanis**.

É assegurada a assistência durante toda pesquisa, bem como me é garantido o livre acesso a todas as informações e esclarecimentos adicionais sobre o estudo e suas conseqüências, enfim, tudo o que eu queira saber antes, durante e depois da minha participação.

Enfim, tendo sido orientado quanto ao teor de todo o aqui mencionado e compreendido a natureza e o objetivo do já referido estudo, manifesto meu livre consentimento em participar, estando totalmente ciente de que não há nenhum valor econômico, a receber ou a pagar, por minha participação.

No entanto, caso eu tenha qualquer despesa decorrente da participação na pesquisa, haverá ressarcimento na forma seguinte: o ressarcimento será em dinheiro, ou mediante depósito em conta-corrente ou cheque. De igual maneira, caso ocorra algum dano decorrente da minha participação no estudo, serei devidamente indenizado, conforme determina a lei.

Em caso de reclamação ou qualquer tipo de denúncia sobre este estudo devo ligar para o CEP PUCPR (41) 3271-2292 ou mandar um email para "nep@pucpr.br".

Curitiba, _____ de _____ de 20____.

Nome e assinatura do participante da pesquisa

Nome(s) e assinatura(s) do(s) pesquisador(es) responsável(responsáveis)

RUBRICA DO SUJEITO DE PESQUISA

RUBRICA DO PESQUISADOR

Parecer Da Revista Drug And Alcohol Dependence

De: eesserver@eesmail.elsevier.com <eesserver@eesmail.elsevier.com> em nome de Lin Lu <eesserver@eesmail.elsevier.com>

Enviado: segunda-feira, 3 de junho de 2019 11:23
Para: Luciana Reis Azevedo Alanis; lrazevedo@yahoo.com
Assunto: Your submission: LL-19-0788R2

Ref.: Ms. No. LL-19-0788R2
Salivary proteome characterization of alcohol and tobacco dependents.
Drug and Alcohol Dependence

Dear Professor Luciana Azevedo-Alanis,
I am pleased to inform you that your submission to Drug and Alcohol Dependence has now addressed all of the issues that were raised in the review process. Thank you for your careful responses to the questions raised by the reviewers and myself. Congratulations again on this interesting and important paper.

The manuscript is being forwarded to the Central Editorial Office with my recommendation that it be published. If there are no further changes that need your attention, the manuscript will promptly be sent on to Elsevier Science Ireland, Ltd. where it will be prepared for publication. You will be alerted by email about the availability of page proofs and about any other actions needed from you. Please respond to such emails as quickly as you can to avoid delays in the appearance of your paper in the journal. After you receive communication from Elsevier, you will be able to follow the status of your paper on the Internet by logging on and registering with the Elsevier (http://www.elsevier.com/authors) which provides additional services, such as Contents Direct that sends to you the tables of contents of your selected journals.

Thank you for submitting your work to this journal.
With kind regards,
Lin Lu, M.D., Ph.D
Associate Editor
Drug and Alcohol Dependence

Material Suplementar

Expression differences			Protein	Score	Accession Number	Biological process[a]	Molecular function[b]	Cellular component[c]
Alcoholic	Control	Ratio A/C						
+	-		116 kDa U5 small nuclear ribonucleoprotein component	784.65	Q15029	1, 3, 4, 5, 9	20, 21, 22, 23, 24	43, 44, 45, 46, 47, 48
+	-		2-(3-amino-3-carboxypropyl) histidine synthase subunit 2	490.09	E9PPU3	-	-	-
+	-		26S proteasome regulatory subunit 4	409.61	P62191	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15	20, 21, 22, 23, 24	43, 44, 45, 46, 47
+	-		ABI gene family member 3 (NESH) binding protein isoform CRA_d	376.55	Q5JPC9	-	-	-
+	-		Adhesion G protein-coupled receptor L4	493.14	Q9HBW9	1, 2, 4, 8	20, 21, 29	43, 44, 45, 46
+	-		Adhesion G-protein-coupled receptor G5	227.59	A0A087WZA0	-	-	-
+	-		A-kinase anchor protein 13	236.4	Q12802	1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 15	20, 21, 22, 25, 29, 30, 31	43, 44, 45, 46, 47
+	-		Alpha-enolase	194.01	P06733	1, 2, 3, 4, 5, 6, 7, 8, 9, 13, 14, 15	20, 21, 22, 23, 34	43, 44, 45, 46, 47, 48, 49, 51
+	-		Anion exchange protein	289.74	E7EQT3	-	-	-
+	-		Annexin A1	286.47	P04083	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 17	20, 21, 22, 23, 24, 27, 33, 35	43, 44, 45, 46, 47, 48, 49, 51
+	-		Apolipoprotein C-II	560.74	V9GZ01	-	-	-
+	-		Apoptosis-inducing factor 2	418.64	Q9BRQ8	1, 2, 3, 15	20, 23,41	43, 44, 45, 46, 48, 49
+	-		Apoptotic protease-activating factor 1	244.17	O14727	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 14, 15, 17	20, 21, 27	43, 44, 45, 47, 48, 49
+	-		Armadillo repeat-containing protein 10	235.04	Q8N2F6	2	-	43, 44, 45, 46
+	-		Ataxin-3	243.55	G3V526	-	-	-
+	-		ATP-dependent RNA helicase DDX39A	263.25	O00148	1, 2, 3, 4, 5, 7, 9, 10	20, 21, 22, 23, 24, 35	43, 44, 45, 46, 47
+	-		Beta-galactosidase (Fragment)	155.03	C9J4G9	-	-	-
+	-		Beta-secretase 1	135.97	P56817	1, 2, 3, 5, 8, 14	20, 21, 23, 24	43, 44, 45, 46, 51

+	-	Breast cancer type 1 susceptibility protein	488.34	E9PC22	-	-	-
+	-	BRISC and BRCA1-A complex member 2	292.92	Q9NXR7	1, 2, 3, 4, 5, 8, 9, 15	20, 21	43, 44, 47
+	-	Butyrophilin subfamily 3 member A2	172.21	P78410	1, 4, 6, 10, 17	-	43, 46
+	-	Cadherin-2	286.97	P19022	1, 2, 3, 4, 5, 6, 8 10, 11, 16	20, 21	43, 44, 45, 46, 48, 49, 51
+	-	Calcium-binding and coiled-coil domain-containing protein 2	329.09	Q13137	1, 2, 3, 4, 13, 14	20, 21	43, 44, 45, 46, 47
+	-	Calcium-binding protein 2	401.36	A0A1B0GW24	-	-	-
+	-	Calmodulin-like protein 3	497.85	P27482	-	20, 21	48, 49
+	-	Cancer/testis antigen 1	229.92	P78358	-	20, 21	43, 44, 45
+	-	CASP8 and FADD-like apoptosis regulator	246.14	O15519	1, 2, 3, 4, 5, 6, 7, 8, 9, 11 12, 13, 15, 19	20, 21, 23, 24, 27	43, 44, 45, 46
+	-	Catenin delta-2	314.48	Q9UQB3	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12,	20, 21	43, 44, 45, 47
+	-	CDK5 regulatory subunit-associated protein 1	329.18	Q96SZ6	1, 2, 3, 5, 6, 7, 9, 11, 12	20, 21, 23, 26	43, 44, 45
+	-	Cell cycle and apoptosis regulator protein 2	436.7	Q8N163	1, 2, 3, 4, 5, 7, 8, 9, 14, 15	20, 21, 22, 27	43, 44, 45, 47, 50
+	-	CG9886-like_ isoform CRA_a	186.7	C9J3N5	-	-	-
+	-	CG9886-like_ isoform CRA_f	186.7	C9JA32	-	-	-
+	-	Chloride channel protein 2	419.87	P51788	1, 2, 6, 10, 11, 12	25, 28, 30	43, 46
+	-	Chromosome 12 open reading frame 75	349.33	F8VXK5	-	-	-
+	-	Chymotrypsin-like elastase family member 2B (Fragment)	284.11	Q5JRU4	-	-	-
+	-	Cofilin-1	376.71	P23528	1, 2, 3, 4, 5, 6, 8, 11, 12, 13, 15, 16	20, 21	43, 44, 45, 46, 47, 48, 49
+	-	Coiled-coil domain-containing protein 129	387.17	Q6ZRS4	-	20, 21	-
+	-	Complement C1q tumor necrosis factor-related protein 7	266.44	Q9BXJ2	-	-	48
+	-	CPX chromosomal region candidate gene 1 protein	159.37	Q8N123	-	20, 22	-
+	-	Crossover junction endonuclease MUS81	213.01	Q96NY9	1, 2, 3, 4, 5, 7, 9, 14	20, 21, 22, 23, 24	43, 44, 47

+	-	Cystatin-8	287.69	O60676	1, 2, 3, 5	20, 21, 27	43, 44, 45, 48, 49, 51
+	-	Cytochrome P450 2F1 (Fragment)	149.53	A0A075B795	-	-	-
+	-	DENN domain-containing protein 1C	254.06	Q8IV53	-	20, 21	43, 44, 45
+	-	Desmocollin-1	155.63	Q08554	1, 4, 6, 10, 11, 12, 15, 17	20	43, 44, 45, 46, 48, 49
+	-	Disks large homolog 4 (Fragment)	321.75	K7EQM6	-	-	-
+	-	DmX-like protein 2	883.22	Q8TDJ6	1	20, 21	43, 44, 45, 46, 48, 49
+	-	DNA excision repair protein ERCC-6	328.5	Q03468	1, 2, 3, 4, 5, 6, 7, 8, 9, 15	20, 21, 22, 23, 24, 27	43, 44, 47
+	-	DNA mismatch repair protein Mlh3	187.07	Q9UHC1	1, 3, 4, 5, 6, 9, 13	20, 21, 22, 23, 24	43, 44, 47, 50
+	-	DNA polymerase-transactivated protein 6_ isoform CRA_b	431.69	B8ZZZ7	-	-	-
+	-	DNA repair protein XRCC1	552.8	P18887	1, 2, 3, 4, 5, 6, 7, 9, 11	20, 21, 22, 23, 24, 32	43, 44, 47, 50
+	-	Double homeobox protein 4C	296.83	Q6RFH8	1, 2, 3, 5, 7, 9	20, 21, 22	43, 44, 47
+	-	Dual 3'_5'-cyclic-AMP and -GMP phosphodiesterase 11A	188.26	Q9HCR9	1, 2, 3, 4, 8	20, 23, 24	43, 44, 45
+	-	E3 ubiquitin-protein ligase HERC2	342.82	O95714	1, 3, 4, 5, 6, 9, 10, 13, 14	20, 21, 22, 25	43, 44, 45, 46, 47
+	-	E3 ubiquitin-protein ligase Mdm2	305.93	F5GWH7	-	-	-
+	-	Electrogenic sodium bicarbonate cotransporter 4	489.96	Q9BY07	1, 2, 3, 5, 6, 11, 17	25, 28	43, 46
+	-	Endoplasmic reticulum membrane-associated RNA degradation protein	318.74	Q5T6L9	6, 11	-	43, 44, 45, 46
+	-	Endothelin-3	441.03	P14138	1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 16, 17	20, 21	43, 44, 48, 49
+	-	Enolase-phosphatase E1	295.13	Q9UHY7	1, 3, 8	20, 22, 24	43, 44, 45, 47, 48, 49
+	-	ERI1 exoribonuclease 3	332.99	O43414	1, 3, 5, 9	20, 22, 23, 24	-
+	-	Eukaryotic initiation factor 4A-I	176.57	P60842	1, 2, 3, 5, 7, 9, 13, 14	20, 21, 22, 23, 24, 35	43, 44, 45, 46, 48, 49
+	-	Fatty acid desaturase 2	957.32	F5GZU2	-	-	-
+	-	Gamma-tubulin complex component (Fragment)	183.53	A0A087WVZ8	-	-	-

+	-	Geranylgeranyl pyrophosphate synthase	469.4	O95749	1, 2, 3, 7	20, 21, 23, 26	43, 44, 45
+	-	Glycine dehydrogenase (decarboxylating) mitochondrial	214.35	P23378	1, 3, 4, 14	20, 21, 23, 34, 36, 41	43, 44, 45, 46, 47
+	-	Glycosylphosphatidylinositol anchor attachment 1 protein	321.48	E9PLV6	-	-	-
+	-	Golgin subfamily A member 2	189.6	Q08379	1, 2, 3, 4, 7, 10, 14	20, 21	43, 44, 45, 46
+	-	G-protein coupled receptor 182	248.28	O15218	1, 2, 4, 8	29	43, 44, 46
+	-	Heat shock protein HSP 90-alpha A2	263.65	Q14568	1, 4	20, 21	43, 44, 45, 48, 49
+	-	Heparan sulfate 2-O-sulfotransferase 1	319.04	Q7LGA3	3, 5, 7	23, 26	43, 44, 45, 46
+	-	Histidine--tRNA ligase_ cytoplasmic	169.5	P12081	1, 3, 5, 7, 9	20, 21, 23, 32	43, 44, 45
+	-	Histone-binding protein RBBP4	473.01	Q09028	1, 2, 3, 4, 5, 7, 8, 9	20, 21, 22, 23, 24	43, 44, 45, 47, 50
+	-	Homeobox protein SIX1	415.34	Q15475	1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 15, 16	20, 21, 22	43, 44, 45, 47
+	-	Integrin alpha-5	743.94	P08648	1,2, 3, 4, 5, 6, 8, 11, 12, 13, 15, 16, 18, 19	20, 21	43, 44, 45, 46, 51
+	-	Intercellular adhesion molecule 2	314.29	P13598	1, 2, 4, 8, 19	20, 21	43, 46, 48, 49
+	-	Interferon gamma receptor 2	193.44	P38484	1, 2, 4, 8, 13	29	43, 44, 45, 46
+	-	Interferon lambda-4	489.3	K9M1A9	-	-	-
+	-	Interleukin-17 receptor D	194.78	Q8NFM7	1, 2, 3, 4, 5, 8	29	43, 44, 45, 46, 47
+	-	Keratin-associated protein 12-1	315.13	P59990	1, 6, 11, 12	20, 21	43, 44, 45
+	-	Killer cell immunoglobulin-like receptor 2DL1	529.02	P43626	1, 2, 4, 8	20, 21	43, 46
+	-	Kinectin	920.29	Q86UP2	1, 3, 5, 16	20, 21, 22	43, 44, 45, 46
+	-	KIR2DL1	229.54	Q6H2H3	-	-	-
+	-	Kv channel-interacting protein 4	238.17	Q6PIL6	1, 2, 6, 10	20, 21, 25, 28, 30	43, 44, 45, 46
+	-	Laminin subunit alpha-1	177.89	P25391	1, 2, 4, 6, 8, 11, 16, 19	20, 21, 33	43, 46, 48, 49, 52
+	-	La-related protein 1	79.41	Q6PKG0	1, 2, 3, 4, 5, 7, 8, 9, 13, 14	20, 21, 23, 42	43, 44, 45, 46

+	-	Long-chain fatty acid transport protein 3	203.4	Q5K4L6	1, 3	20, 22, 34	43, 44, 45, 46
+	-	MAGUK p55 subfamily member 2 (Fragment)	561.1	E5RK50	-	-	-
+	-	MAGUK p55 subfamily member 6	471.96	Q9NZW5	-	20, 21	43, 46, 48, 49
+	-	MAL-like protein	454.44	C9IZ55	-	-	-
+	-	Mediator of RNA polymerase II transcription subunit 4 (Fragment)	219.05	Q5T911	-	-	-
+	-	Metalloproteinase inhibitor 2	359.11	P16035	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 17, 19	20, 21, 27	43, 44, 45, 48, 49, 51, 52
+	-	Methionine adenosyltransferase 2 subunit beta	806.71	E5RJR3	-	-	-
+	-	Mitochondrial import inner membrane translocase subunit Tim9	869.02	Q9Y5J7	1, 6, 10	20, 21, 25, 38	43, 44, 45, 46
+	-	Multidrug resistance-associated protein 6	366.74	O95255	4, 6, 10	20, 23, 24, 25, 28	43, 44, 45, 46, 47
+	-	Myelin regulatory factor	189.78	Q9Y2G1	1, 2, 3, 5, 6, 7, 9, 11, 12	20, 22, 23, 24	43, 44, 45, 46, 47
+	-	Natural resistance-associated macrophage protein 2	560.21	P49281	1, 3, 4, 6, 7, 10, 11, 12, 18	20, 21, 25, 28	43, 44, 45, 46, 47, 48, 51
+	-	Neuromedin-U	251.14	A0A0B4J202	-	-	-
+	-	NFU1 iron-sulfur cluster scaffold homolog mitochondrial (Fragment)	580.32	F8W9P7	-	-	-
+	-	Nuclear pore glycoprotein p62 (Fragment)	281.39	M0QX13	-	-	-
+	-	Nuclear protein 1	582.58	H3BS92	-	-	-
+	-	Numb-like protein (Fragment)	485.39	M0QXQ4	-	-	-
+	-	OCIA domain-containing protein 1	379.53	Q9NX40	1, 2, 12	20, 21	43, 44, 45, 46
+	-	Olfactory receptor 52M1	280.57	Q8NGK5	1, 2, 4, 6, 8	29	43, 46
+	-	Oxysterols receptor LXR-alpha	306.8	Q13133	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 17	20, 21, 23, 29	43, 44, 47, 50
+	-	Period circadian protein homolog 2	333.77	O15055	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 17	20, 21, 23	43, 44, 45, 47
+	-	Peripherin	235.9	P41219	-	20, 21, 33	43, 44, 46, 48, 49
+	-	PHD finger protein 21A (Fragment)	263.08	H0YCM5	-	-	-

+	-	Phospholipase B1 membrane-associated (Fragment)	162.51	H7C012	-	-	-
+	-	Phospholipase D3	289.47	Q8IV08	3, 14	20, 21, 22, 24	43, 44, 45, 46, 48, 49
+	-	Pigment epithelium-derived factor	224.75	P36955	1, 2, 3, 4, 5, 6, 11, 12, 15, 16, 18	20, 21, 26	43, 44, 45, 48, 49, 52
+	-	Plexin-B2	178.7	O15031	1, 2, 3, 4, 5, 6, 8, 11, 12, 16	20, 21, 29	43, 46, 48, 49, 51
+	-	Profilin-1	395.5	P07737	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 16	20, 21, 23, 26	43, 44, 45, 46, 47, 48, 49
+	-	Programmed cell death 6-interacting protein	609.3	Q8WUM4	1, 2, 3, 5, 10, 13, 14, 15, 17, 19	20, 21	43, 44, 45, 46, 48, 49
+	-	Prolyl 3-hydroxylase OGFOD1 (Fragment)	685.13	H3BR79	-	-	-
+	-	Prominin-1	744.73	O43490	1, 2, 6, 11, 12	20, 21	43, 44, 45, 46, 48, 49, 51
+	-	Protein fantom	335.54	Q68CZ1	1, 2, 4, 6, 8, 11	20, 21	43, 44, 45, 46, 47
+	-	Protein limb expression 1 homolog	683.94	Q8N485	1, 3, 14	-	43, 44, 45
+	-	Protein NEDD1	499.57	G3V2S2	-	-	-
+	-	Protein phosphatase Slingshot homolog 1	454.82	Q8WYL5	1, 2, 3, 4, 5, 6, 8, 11, 12, 15, 16	20, 21, 23, 24	43, 44, 45, 46
+	-	Protein PROCA1	391.75	J3QQU2	-	-	-
+	-	Protein PRRC2C	178.79	Q9Y520	1, 6, 11, 12	20, 21, 22	43, 44, 45, 46
+	-	Protein S100-A8	663.77	P05109	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17	20, 21	43, 44, 45, 46, 47, 48, 49
+	-	Protein YIPF2 (Fragment)	221.34	K7ENZ5	-	-	-
+	-	Puromycin-sensitive aminopeptidase	452.87	P55786	1, 2, 3, 4, 5, 10, 14	20, 23, 24	43, 44, 45, 47, 48, 49
+	-	Putative beta-actin-like protein 3	162.39	Q9BYX7	1, 10, 17	20	43, 44, 45, 48, 49
+	-	Putative mitochondrial import inner membrane translocase subunit Tim23B	351.32	S4R2X5	-	-	-
+	-	Putative tetraspanin-19	371.38	P0C672	1, 2, 4, 8	-	43, 46
+	-	Pyruvate kinase PKM	357.47	P14618	1, 3, 4, 6, 7, 9, 10, 11, 14, 15, 17	20, 21, 22, 23, 26, 31	43, 44, 45, 47, 48, 49
+	-	Regulator of G-protein signaling 3	335.9	P49796	-	-	-

+	-	Retinal dehydrogenase 1 (Fragment)	193.18	Q5SYQ8	-	-	-
+	-	Retinoblastoma-associated protein	762.38	P06400	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15	20, 21, 22	43, 44, 47, 50
+	-	Retinol dehydrogenase 10	580.37	Q8IZV5	1, 3, 6, 7, 11, 12	23, 36	43, 44, 45, 46
+	-	Rho GTPase-activating protein 28	400.33	Q9P2N2	1, 2, 4, 8	27	43, 44, 45
+	-	Rho guanine nucleotide exchange factor 15	443.59	O94989	1, 2, 4, 6, 8, 11	20, 21, 27	43, 44, 45
+	-	RING finger protein 157	390.22	Q96PX1	-	-	-
+	-	RNA-binding protein 12B	380.59	Q8IXT5	-	20, 23, 26	43, 44, 45
+	-	Semaphorin-4C	336.17	Q9C0C4	-	20, 21, 22	-
+	-	Serotransferrin	114.86	P02787	1, 2, 3, 4, 5, 6, 10, 17	20, 21, 27, 28	43, 44, 45, 46, 48, 49, 51
+	-	Signal-transducing adaptor protein 2	281.14	Q9UGK3	1,2, 3, 4, 5, 8	20, 21	43, 44, 45, 46
+	-	Solute carrier family 41 member 3	410.58	D6RJC0	-	-	-
+	-	Solute carrier family 45 member 4 (Fragment)	420.15	E5RJM7	-	-	-
+	-	SPATS2-like protein	431.69	Q9NUQ6	-	20, 22	43, 44, 45, 47
+	-	Spermatogenesis-associated serine-rich protein 2	345.93	Q86XZ4	-	20, 22	43, 44, 45
+	-	Sphingosine kinase 2	336.32	Q9NRA0	1, 2, 3, 4, 6, 7, 8, 11, 13, 15	20, 21, 23, 26, 29, 31	43, 44, 45, 46
+	-	Splicing factor 3B subunit 1	375.8	O75533	1, 2, 3, 5, 9	20, 21, 22	43, 44, 47
+	-	Spondin-2	281.23	Q9BUD6	1, 2, 4, 6, 10, 11, 12, 13, 16	20, 21	48, 49, 52
+	-	Stromal cell-derived factor 2	183.12	Q99470	1, 3, 4, 5, 7, 14	22, 25	43, 44, 45, 46, 48, 49
+	-	Surfactant-associated protein 3	531.35	P0C7M3	1, 3, 5	-	43, 44, 45, 46, 48
+	-	Target of Nesh-SH3	366.77	Q7Z7G0	1, 2, 19	20	48, 49, 52
+	-	TBC1 domain family member 17	90.5	Q9HA65	1, 2, 3, 10, 14	20, 21, 27	43, 44, 45
+	-	Trafficking protein particle complex subunit 5	219.03	Q8IUR0	1, 10	20, 21	43, 44, 45, 46

+	-	Transcription factor SOX-13	266.5	Q9UN79	1, 2, 3, 5, 6, 7, 9, 11, 12	20, 21, 23	43, 44, 47
+	-	Transcription regulator protein BACH2	215.36	Q9BYV9	1, 2, 3, 5, 7, 9, 10	20, 22, 23, 26	43, 44, 45, 47
+	-	Transforming growth factor beta receptor type 3	508.26	Q03167	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 16	20, 21, 23, 26, 29, 31	43, 44, 45, 46, 48, 49, 51, 52
+	-	Type 2 lactosamine alpha-2_3-sialyltransferase	702.63	Q9Y274	1, 3, 4, 5, 7	23, 26	43, 44, 45, 46, 48, 49
+	-	Tyrosine-protein phosphatase non-receptor type 1	314.56	P18031	1, 2, 3, 4, 5, 8, 9, 14, 15	20, 21, 22, 23, 24	43, 44, 45, 46
+	-	U4/U6.U5 tri-snRNP-associated protein 1	197.66	O43290	1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 15	20, 21, 22	43, 44, 45, 47
+	-	Ubiquitin carboxyl-terminal hydrolase 7	967.93	Q93009	1, 2, 3, 4, 5, 6, 7, 99, 11, 13, 14	20, 21, 22, 24	43, 44, 45, 47, 50
+	-	Uncharacterized protein (Fragment)	859.44	M0R2N6	1, 3, 4, 5, 9	20, 23	43, 44, 47
+	-	Uncharacterized protein (Fragment)	448.02	K7ERI5	1, 2, 3, 5, 7, 9	20, 23	43, 44
+	-	Uncharacterized protein (Fragment)	301.82	D6RBZ9	-	-	-
+	-	Uncharacterized protein C3orf36	242.9	Q3SXR2	-	-	-
+	-	Uncharacterized protein FLJ43738	361.59	Q6ZUG5	-	-	-
+	-	Uncharacterized protein KIAA0895-like (Fragment)	289.03	I3L230	-	-	-
+	-	Uncharacterized protein KIAA0895-like	318.33	Q68EN5	-	-	-
+	-	Uncharacterized protein	557.93	A0A1W2PRE2	-	-	-
+	-	Uncharacterized protein	377.68	A0A1W2PQF6	-	-	-
+	-	Uncharacterized protein	270.86	A0A1W2PQZ7	-	-	-
+	-	Voltage-dependent calcium channel gamma-1 subunit	239.22	Q06432	2, 6, 10	20, 21, 25, 28, 30	43, 46
+	-	WW domain-containing adapter protein with coiled-coil	187.05	Q9BTA9	1, 2, 3, 4, 5, 7, 9, 14	20, 21	43, 44, 47
+	-	Xaa-Pro dipeptidase	512.85	P12955	1, 3, 5, 14	20, 21, 23, 24	43, 44, 47, 48, 49
+	-	Zinc finger and SCAN domain-containing protein 31 (Fragment)	319.17	C9J6S7	-	-	-
+	-	Zinc finger protein 112	464.21	Q9UJU3	1, 2, 3, 5, 7, 9	20, 22	43, 44, 47

+	-	Zinc finger protein 155	268.9	Q12901	1, 2, 3, 5, 7, 9	20, 22	43, 44, 47
+	-	Zinc finger protein 177	268.9	Q13360	1, 2, 3, 5, 7, 9	20, 21, 22	43, 44, 47, 48, 49
+	-	Zinc finger protein 221	268.9	Q9UK13	1, 2, 3, 5, 7, 9	20, 22	43, 44, 47
+	-	Zinc finger protein 222	268.9	Q9UK12	1, 2, 3, 5, 7, 9	20, 22	43, 44, 47
+	-	Zinc finger protein 223	282.6	Q9UK11	1, 2, 3, 5, 7, 9	20, 21, 22	43, 44, 47
+	-	Zinc finger protein 224	268.9	Q9NZL3	1, 2, 3, 5, 7, 9	20, 21, 22	43, 44, 46, 47
+	-	Zinc finger protein 235	614.72	Q14590	1, 2, 3, 5, 7, 9	20, 22	43, 44, 47
+	-	Zinc finger protein 284	268.9	Q2VY69	1, 2, 3, 5, 7, 9	20, 22	43, 44, 47
+	-	Zinc finger protein 333	153.96	M0R113	-	-	-
+	-	Zinc finger protein 45	268.9	Q02386	1, 2, 3, 5, 6, 7, 9, 11	20, 22	43, 44, 47
+	-	Zinc finger protein 850	475.3	A8MQ14	1, 2, 3, 5, 7, 9	20, 22	43, 44, 47
+	-	Zinc finger protein 98 (Fragment)	254.29	M0R243	-	-	-
+	-	Zinc finger protein with KRAB and SCAN domains 2	209.23	Q63HK3	1, 2, 3, 5, 7, 9	20, 22	43, 44, 47
+	-	ZNF559-ZNF177 readthrough (Fragment)	268.9	S4R3Q2	-	-	-
-	+	Alanine aminotransferase 2	182.21	Q8TD30	1, 3, 7, 14	20, 22, 25	43, 44, 45
-	+	Alpha-1_3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase A	562.44	Q9UM21	1, 3, 5, 7	20, 22, 25	43, 44, 45, 46, 48, 49
-	+	Amino acid transporter	304.12	E7EV13	-	-	-
-	+	Aminomethyltransferase_ mitochondrial	462.23	A0A1B0GU55	-	-	-
-	+	Amyloid-like protein 1	464.22	P51693	1, 2, 4, 6, 8, 10, 11, 15	20, 21	43, 44, 45, 46, 48, 52
-	+	Androglobin	263.77	Q8N7X0	3, 5	22, 24	43, 44, 45
-	+	ATP-binding cassette sub-family F member 3	319.53	Q9NUQ8	4, 13	20, 21, 22, 24	43, 46
-	+	AT-rich interactive domain-containing protein 1A	230.61	O14497	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	20, 21, 23	43, 44, 47, 50

-	+	BPI fold-containing family B member 2	1006.56	Q8N4F0	1, 3, 4, 5, 13	20	43, 44, 45, 49
-	+	Cadherin-9	259.91	Q9ULB4	1, 6, 11	20	43, 46
-	+	Calcyclin-binding protein	324.05	Q9HB71	1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 15	20, 21	43, 44, 45, 47, 48, 49
-	+	Calpastatin	441.46	P20810	1, 2, 3, 5, 15	20, 21, 23, 26	43, 44, 45, 46
-	+	Carboxypeptidase D (Fragment)	246.16	J3QRJ9	-	-	-
-	+	Cell adhesion molecule-related/down-regulated by oncogenes	601.39	Q4KMG0	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12	20, 21	43, 46, 48
-	+	Coiled-coil alpha-helical rod protein 1	295.37	Q8TD31	1, 6, 10, 11, 12	-	43, 44, 45, 47
-	+	Condensin complex subunit 1	463.92	F5H431	-	-	-
-	+	Cystatin-D	390.69	P28325	1, 2, 3, 5	20, 21, 26	48, 49
-	+	Cytosolic arginine sensor for mTORC1 subunit 1	241.45	Q8WTX7	1, 2, 4, 8	20, 21	43, 44, 45
-	+	DCN1-like protein 3	315.55	Q8IWE4	1, 2, 3, 4, 5, 15	20, 21	43, 44, 45, 46
-	+	Deoxyribonuclease gamma	150.4	Q13609	1, 2, 3, 4, 5, 9, 12, 14, 15	20, 22, 23, 24	43, 44, 45, 47, 48
-	+	Deoxyribonuclease-1-like 1	1170.24	P49184	1, 3, 4, 5, 9, 10, 14, 17	20, 22, 23, 24	43, 44, 45, 47, 48, 49
-	+	DNA replication licensing factor MCM2	360.93	P49736	1, 3, 4, 5, 6, 7, 9, 11, 15	20, 21, 23, 24, 36	43, 44, 45, 47, 50
-	+	Double-strand-break repair protein rad21 homolog	291.83	E5RI01	-	-	-
-	+	Dynamin-1 (Fragment)	828.61	A0A1B0GVK6	-	-	-
-	+	EH domain-binding protein 1	326.75	Q8NDI1	10	-	43, 44, 45, 46
-	+	Erbin	178.98	Q96RT1	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13	20, 22, 31	43, 44, 45, 46, 47, 48, 52
-	+	Espin	185.71	B1AK53	1, 2, 4, 6, 18	20, 21	43, 44, 45
-	+	Excitatory amino acid transporter 4 (Fragment)	289.84	M0R2V7	-	-	-
-	+	F-BAR domain only protein 2	431.81	Q0JRZ9	1, 10	20, 21	43, 44, 45, 46
-	+	Focadhesin	215.38	Q5VW36	-	-	-

-	+	Galactoside 2-alpha-L-fucosyltransferase 1	311.95	P19526	1, 3, 5, 7, 14	22, 25	43, 44, 45, 46
-	+	Galectin-9	1829.23	O00182	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17	20, 21, 29	43, 44, 45, 47, 48, 49
-	+	Glutamate--cysteine ligase catalytic subunit	175.08	P48506	1, 2, 3, 4, 5, 6, 7, 8, 9, 14, 15	20, 21, 22, 34	43, 44, 45
-	+	Graves' disease carrier protein	1021.57	P16260	1, 3, 7, 10	27	43, 44, 45, 46
-	+	GTP-binding nuclear protein Ran	571.65	A0A087X0W0	-	-	-
-	+	HCG1651889_ isoform CRA_d (Fragment)	213.97	C9JVX5	-	-	-
-	+	HEAT repeat-containing protein 5A	470.79	Q86XA9	-	-	-
-	+	Hematopoietic prostaglandin D synthase	236.86	O60760	1, 2, 3, 4, 6, 7, 8, 13, 18	20, 21, 22, 25, 39	43, 44, 45
-	+	Histone deacetylase 6	559	Q9UBN7	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16	20, 21, 23, 24	43, 44, 45, 46, 47
-	+	Homeobox protein CDX-1	209.61	P47902	1, 2, 3, 5, 7, 8, 9, 11, 12, 13	20, 21, 23	43, 44, 47
-	+	Immunoglobulin heavy variable 3/OR16-9 (non-functional)	519.84	S4R460	-	-	-
-	+	Immunoglobulin kappa constant	652.11	P01834	1, 2, 3, 4, 5, 6, 8, 10, 13, 16	20, 21, 22, 24	43, 46, 48, 49, 51
-	+	Interleukin 15 receptor alpha isoform EM2	380.51	K9N2S2	-	-	-
-	+	Interleukin-15 receptor subunit alpha	396.53	Q13261	1, 2, 4, 8	20, 21, 29	43, 44, 45, 46, 47, 48, 49
-	+	LARGE xylosyl- and glucuronyltransferase 1 (Fragment)	486.49	B0QZ00	-	-	-
-	+	Leucine-rich repeat-containing protein 2	189.97	Q9BYS8	-	-	-
-	+	Low affinity immunoglobulin gamma Fc region receptor III-B	257.24	O75015	1, 3, 4, 5, 7, 10, 17	20	43, 44, 45, 46, 48, 49
-	+	Lysine-specific demethylase 5D	196.49	Q9BY66	1, 2, 3, 4, 5, 7, 9	20, 21, 22, 23, 32	43, 44, 47
-	+	Lysophosphatidylcholine acyltransferase 1	140.32	Q8NF37	1, 2, 3, 4, 5, 6, 7, 10, 11, 14, 17	20, 22, 25	43, 44, 45, 46
-	+	Matrix metalloproteinase-19	370.87	Q99542	1, 3, 4, 5, 6, 11, 12, 13, 14, 19	20, 22, 24	48, 49, 52
-	+	Methionine--tRNA ligase_ cytoplasmic (Fragment)	347.96	H0YI27	-	-	-
-	+	Mucin-7	1208.62	Q8TAX7	1, 2, 3, 4, 5, 7, 8, 13	20, 21	43, 44, 45, 46, 48, 49

-	+	Multiple PDZ domain protein	257.5	O75970	13	20, 21	43, 44, 45, 46
-	+	Myc-associated zinc finger protein	191.61	P56270	-	20, 21, 23	43, 44, 47
-	+	NACHT_ LRR and PYD domains-containing protein 7	257.84	Q8WX94	1, 2, 3, 4, 5, 6, 10, 13, 17	20, 21, 26	-
-	+	Neuroblast differentiation-associated protein AHNAK	600.24	E9PJC6	-	-	-
-	+	Peripheral plasma membrane protein CASK	173.35	O14936	1, 2, 3, 4, 5, 7, 8, 9, 17	20, 21, 22, 25, 30	43, 44, 45, 46, 47, 48, 52
-	+	Peroxisomal targeting signal 1 receptor	391.99	P50542	1, 2, 3, 5, 10	20, 21	43, 44, 45, 46
-	+	Poly [ADP-ribose] polymerase 11	305.53	Q9NR21	1, 6, 10, 12, 13	20, 21, 22, 25	43, 44, 47
-	+	Proline-_ glutamic acid- and leucine-rich protein 1	689.53	Q8IZL8	1, 2, 3, 4, 5, 7, 9	20, 21, 23	43, 44, 45, 47, 50
-	+	Proline-rich protein 23D1	372.4	E9PI22	-	-	-
-	+	Proline-rich protein 23D2	372.4	P0DMB1	-	-	-
-	+	Proline-rich transmembrane protein 4	287.51	C9JH25	-	-	43, 46
-	+	Protein GPR108	435.12	Q9NPR9	-	-	43, 46
-	+	Protein LEG1 homolog	476.9	Q6P5S2	6, 11	-	48, 49
-	+	Protein quaking	252.09	Q96PU8	1, 2, 3, 5, 6, 7, 9, 10, 11, 12 ,13, 14	20, 21, 23	43, 44, 45, 47
-	+	Protein SON	871.13	P18583	1, 2, 3, 5, 9, 15	20, 23, 32	43, 44, 47
-	+	Protein Wiz	207.45	O95785	1, 2, 3, 5, 7, 9	20, 21, 23	43, 44, 47, 48, 49
-	+	Protocadherin alpha-7	181.52	Q9UN72	1, 6, 8, 11	20, 21	43, 46
-	+	Putative ubiquitin carboxyl-terminal hydrolase 17-like protein 23	214.04	D6RBM5	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Rab proteins geranylgeranyltransferase component A 2	348.33	P26374	1, 2, 3, 4, 5, 8, 10	20, 21, 26	43, 44, 45, 47
-	+	Ras-related protein Rab-44	155.37	A0A087WXI0	-	-	-
-	+	Rho-related GTP-binding protein RhoQ (Fragment)	566.62	E5RFZ3	-	-	-
-	+	RWD domain-containing protein 4	693.96	Q6NW29	-	-	-

-	+	Scm-like with four MBT domains protein 1	1092.63	Q9UHJ3	1, 2, 3, 5, 6, 7, 9, 11, 12	20, 32	43, 44, 47
-	+	Serine hydrolase-like protein 2	518.59	Q9H4I8	-	22, 24	43, 44, 45
-	+	Serine hydrolase-like protein	518.59	Q9NQF3	-	22, 24	-
-	+	Serine incorporator 5	206.81	A0A0J9YYI4	-	-	-
-	+	Spermatogenesis-associated protein 6	964.75	Q9NWH7	1, 6, 11, 12, 13	20, 21	43, 48
-	+	Synaptogyrin-3 (Fragment)	278.81	H3BNA6	-	-	-
-	+	Syndecan-1	880.06	P18827	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 14, 16, 17, 19	20, 21	43, 44, 45, 46, 48, 49, 51
-	+	TATA box-binding protein-associated factor RNA polymerase I subunit C	598.33	Q15572	1, 2, 3, 5, 7, 9	20, 21, 23	43, 44, 47
-	+	Trans-3-hydroxy-L-proline dehydratase	171.54	Q96EM0	-	20, 22, 35	-
-	+	Transcription factor 4 (Fragment)	687.53	A0A0E3D6N2	-	-	-
-	+	Transducin-like enhancer protein 3	228.04	Q04726	1, 2, 3, 4, 5, 6, 7, 8, 9, 11	20, 21	43, 44, 47
-	+	Transducin-like enhancer protein 4	311.92	Q04727	1, 2, 3, 4, 5, 7, 8, 9	20, 21	43, 44, 47
-	+	Transient receptor potential cation channel subfamily V member 6 (Fragment)	1641.19	C9JHY1	-	-	-
-	+	Transmembrane protein 107	297.56	Q6UX40	1, 6, 11	20, 21	43, 46
-	+	Transmembrane protein 121	210.97	Q9BTD3	-	-	43, 46
-	+	Transmembrane protein 18	539.2	Q96B42	1, 3, 5, 7, 9, 16	20, 23	43, 44, 45, 46, 47
-	+	Trinucleotide repeat-containing gene 6A protein	377.93	Q8NDV7	1, 2, 3, 4, 5, 6, 7, 8, 11, 12	20, 21, 23	43, 44, 45, 47
-	+	Tumor necrosis factor ligand superfamily member 10	312.36	P50591	1, 2, 3, 4, 5, 6, 8, 11, 15	20, 21	43, 46, 48, 49
-	+	Tyrosine-protein kinase Fer	660.56	P16591	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 16	20, 21, 22, 25, 30	43, 44, 45, 46, 47, 50
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 11	224.55	C9JVI0	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 12	224.55	C9JPN9	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 13	224.55	C9JLJ4	1, 3, 5, 14	22, 24	43, 44, 45, 47

-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 15	224.55	C9J2P7	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 17	224.55	D6RBQ6	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 18	224.55	D6R9N7	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	20, 21, 23	43, 44, 47, 50
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 19	224.55	D6RCP7	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 20	224.55	D6RJB6	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 21	224.55	D6R901	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 22	224.55	D6RA61	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 24	224.55	Q0WX57	1, 2, 3, 5, 14, 15	20, 22, 23, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 5	229.72	A8MUK1	1, 3, 5, 14, 15	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 17-like protein 6	214.04	Q6QN14	1, 3, 5, 14	22, 24	43, 44, 45, 47
-	+	Ubiquitin carboxyl-terminal hydrolase 40	273.95	Q9NVE5	1, 3, 5, 14	22, 24	-
-	+	Ubiquitin-conjugating enzyme E2 C	322.62	O00762	1, 2, 3, 5, 14	20, 21, 22, 25	43, 44, 45, 46, 47
-	+	Uncharacterized protein (Fragment)	389.05	M0QZ58	-	-	-
-	+	Uncharacterized protein (Fragment)	196.34	H7C1Q1	-	-	-
-	+	UPF0577 protein KIAA1324	477.42	E9PIF6	-	-	-
-	+	Uroplakin-3a	260.68	O75631	1, 6, 10, 11, 12	20, 21	43, 44, 45, 46, 48, 49
-	+	Vacuolar protein sorting-associated protein 53 homolog	349.99	Q5VIR6	10	20, 21	43, 44, 45, 46
-	+	WD repeat-containing protein 25	412.44	Q64LD2	-	20, 21	-
-	+	Zinc finger CCCH domain-containing protein 13	191.97	Q5T200	-	20, 21, 23	43, 44, 47
-	+	Zinc finger protein 197	253.68	O14709	1, 2, 3, 5, 7, 9	20, 23	43, 44, 47
-	+	Zinc finger protein 397	259.07	Q8NF99	-	-	-
-	+	Zinc finger protein 432 (Fragment)	400.52	M0R258	1, 2, 3, 5, 7, 9	20, 21, 23	43, 44, 45, 46, 47

-	+		Zinc finger protein 571	180.55	Q7Z3V5	1, 2, 3, 5, 7, 9	20, 23	43, 44, 47
-	+		Zinc finger protein 582	652.6	Q96NG8	1, 2, 3, 5, 7, 9	20, 23	43, 44, 47
-	+		Zinc finger protein 74	563.82	Q16587	1, 2, 3, 5, 6, 7, 9, 11	20, 23	43, 44, 47
-	+		Zinc transporter ZIP11	331.45	J3QRN3	-	-	-
+ ↑	+	1.93	Actin_ alpha cardiac muscle 1	1740.62	P68032	1, 2, 3, 4, 5, 6, 11, 12, 15, 16	20, 21, 22, 24	43, 44, 45, 46, 48, 49
+ ↑	+	2.12	Actin_ alpha skeletal muscle	1753.68	P68133	1, 2, 3, 4, 5, 6, 11, 12, 16	20, 21, 31	43, 44, 45, 48, 49
+ ↑	+	2.16	Actin_ aortic smooth muscle	1740.62	P62736	1, 2, 3, 4, 5, 6, 11, 12, 13	20, 21	43, 44, 45, 48, 49
+ ↑	+	2.29	Actin_ cytoplasmic 1	4796.52	P60709	1, 2, 3, 4, 5, 6, 8, 10, 11, 16	20, 21, 31	43, 44, 45, 46, 47, 48, 49, 50
+ ↑	+	2.14	Actin_ cytoplasmic 2	4796.52	P63261	1, 2, 4, 6, 8, 10, 12	20, 21, 31	43, 44, 45, 46, 48, 49
+ ↑	+	2.46	Actin_ gamma-enteric smooth muscle	1740.62	P63267	2, 3, 5, 6, 11	20	43, 44, 45, 48, 49
+ ↑	+	1.34	Basic salivary proline-rich protein 1	410.33	P04280	-	-	-
+ ↑	+	1.35	Cystatin-S	4894.05	P01036	1, 2, 3, 4, 5, 6	20, 21, 26	48, 49
+ ↑	+	1.03	Cystatin-SN	3222.1	P01037	1, 2, 3, 4, 5, 6	20, 21, 26	48, 49
+ ↑	+	17.81	Forkhead box protein P2	589.65	O15409	1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 18	20, 21, 23	43, 44, 47
+ ↑	+	1.79	GOLGA4 protein	238.64	Q86W71	-	-	-
+ ↑	+	1.75	Golgin subfamily A member 4	268.95	Q13439	1, 2, 6, 10, 11,12	20, 21	43, 44, 45, 46, 48, 49
+ ↑	+	7.61	HCG1745306_ isoform CRA_a	5534.44	G3V1N2	-	-	-
+ ↑	+	5.05	Hemoglobin subunit alpha	8580.57	P69905	1, 2, 3, 4, 10, 14, 15	20, 21, 22, 32, 38	43, 44, 45, 46, 48, 49
+ ↑	+	10.07	Hemoglobin subunit beta	6003.34	P68871	1, 2, 3, 4, 6, 7, 10, 14, 15, 17	20, 21, 22, 32, 38	43, 44, 45, 48, 49
+ ↑	+	4.06	Hemoglobin subunit delta	1171.27	P02042	4, 6, 10	20, 21	43, 44, 45, 48, 49
+ ↑	+	5.26	Hemoglobin subunit gamma-1	387.65	P69891	4, 6, 10	20	43, 44, 45
+ ↑	+	4.85	Hemoglobin subunit gamma-2	387.65	P69892	4, 6, 10	20	43, 44, 45, 48, 49

+ ↑	+	1.52	Homeobox protein DLX-3	521.61	O60479	1, 2, 3, 5, 6, 7, 9, 11, 12	20, 21, 23	43, 44, 47
+ ↑	+	1.35	Keratin_ type II cytoskeletal 73	432.62	Q86Y46	1, 6, 11, 12, 15	20, 21, 31	43, 44, 45, 47, 48, 49
+ ↑	+	3.03	Leukocyte-associated immunoglobulin-like receptor 1	443.41	Q6GTX8	1, 2, 4, 10, 17	20, 21	43, 44, 45, 46, 48, 49
+ ↑	+	2.77	Lipocalin-1	382.08	P31025	1, 2, 3, 4, 5, 6, 10	20, 21, 26	48, 49
+ ↑	+	1.73	Lysozyme C	1050.7	P61626	-	-	-
+ ↑	+	2.97	POTE ankyrin domain family member E	1170.4	Q6S8J3	1, 3, 4, 5, 6, 10, 13, 14, 17	20, 21, 22, 24	43, 44, 45, 48, 49
+ ↑	+	2.89	POTE ankyrin domain family member F	1170.4	A5A3E0	-	-	-
+ ↑	+	5.53	Voltage-dependent calcium channel subunit alpha-2/delta-3	316.29	Q8IZS8	2, 6, 10	20, 27, 28, 33	43, 46
+ ↑	+	1.03	Cystatin-SA	1324.57	P09228	1, 2, 3, 4, 5, 6	20, 21, 26	48, 49
+ ↑	+	4.53	DENN domain-containing protein 5A	207.1	Q6IQ26	1, 2, 4, 6, 10, 11, 12	20, 21, 27, 28, 33	43, 44, 45, 46
+ ↑	+	1.15	Prolactin-inducible protein	986.28	P12273	1, 2, 3, 4, 5, 6, 10, 15	20, 21, 22, 24	43, 44, 47, 48, 49
+ ↑	+	2.23	Tetraspanin	310.65	A6NNI4	-	-	-
+ ↑	+	1.01	Alpha-amylase 1	27231.79	P04745	3, 6	20, 21, 22, 24	48, 49
+ SE	+	2.23	Ankyrin-3	305.51	Q12955	-	-	-
+ SE	+	1.02	Serum albumin	3074.69	P02768	1, 2, 3, 4, 5, 6, 8, 10, 13, 15, 17, 19	20, 21, 23, 38	43, 44, 45, 47, 48, 49
+ SE	+	1.43	Forkhead box P2_ isoform CRA_a	485.16	Q75MZ5	-	-	-
+ SE	+	1.04	Forkhead box P2 variant 3	494.51	Q0PRL4	-	-	-
+ SE	+	1.03	Protocadherin-15	128.88	Q96QU1	-	-	-
+ SE	+	1.12	Zinc finger E-box-binding homeobox 2	506.33	O60315	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 16	20, 23, 26, 32	43, 44, 45, 47, 50
+ SE	+	1.03	ATP-binding cassette sub-family B member 9	411.65	Q9NP78	10	20, 21, 22, 24, 27	43, 44, 45, 46
+ SE	+	1.03	RNA-binding protein Raly	255.08	Q9UKM9	1, 2, 3, 5, 7, 9	20, 21, 23	43, 44, 47
+ SE	+	1.03	Immunoglobulin lambda variable 6-57	1036.56	P01721	1, 2, 3, 4, 5, 8, 10, 16	20, 22, 24	43, 46, 48, 49

+ SE	+	1.04	Ropporin-1B	160.92	Q9BZX4	1, 2, 3, 4, 5, 6, 8, 12, 13, 16	20, 21, 31	43, 44, 45
+ SE	+	1.04	Ubiquitin-like protein ISG15	274.65	P05161	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13	20, 21	43, 44, 45, 47, 48
+ SE	+	1.06	Protein transport protein Sec23A	190.4	Q15436	1, 10	20, 21	43, 44, 45, 46
+ SE	+	1.84	Hemoglobin subunit epsilon	387.65	P02100	1, 4, 6, 10	20, 21	43, 44, 45, 48, 49
+ SE	+	1.02	Microtubule-associated serine/threonine-protein kinase 4	333.65	O15021	1, 2, 3, 4, 5, 8	20, 22, 25, 30	43, 44, 45
+ SE	+	1.08	Translation initiation factor eIF-2B subunit alpha	255.56	Q14232	1, 3, 4, 5, 6, 7, 11, 12	20, 21, 23	43, 44, 45, 46
+ SE	+	1.04	Kinetochore-associated protein NSL1 homolog	202.78	Q96IY1	1	20, 21	43, 44, 45, 47, 50
+ SE	+	0.97	Olfactory receptor 6C68	193.41	A6NDL8	1, 2, 4, 6, 8	29	43, 46
+ SE	+	1.04	Ras GTPase-activating protein 2	237.38	Q15283	1, 2, 3, 4, 5, 8	20, 26	43, 44, 45, 46
+ SE	+	1.02	Protein unc-13 homolog B	302.1	O14795	1, 2, 4, 6, 8, 10, 11, 13, 15, 17	20, 21	43, 44, 45, 46
+ SE	+	1.03	E3 ubiquitin-protein ligase RNF165	518.87	Q6ZSG1	-	20, 21, 22, 25	43, 44, 45, 47
+ SE	+	1.15	Protein PRR14L	196.81	Q5THK1	-	-	-
+ SE	+	1.04	Uncharacterized protein C8orf31	278.7	Q8N9H6	-	-	-
+ SE	+	1.03	26S proteasome non-ATPase regulatory subunit 11	197.98	O00231	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 17	20, 21, 31	43, 44, 45, 46, 47, 48, 49
+ SE	+	1.03	Transforming growth factor-beta-induced protein ig-h3	348.26	Q15582	-	-	-
+ SE	+	1.06	Zinc finger protein 575	250.34	Q86XF7	1, 2, 3, 4, 5, 6, 11, 12, 19	20, 21	43, 44, 45, 46, 48, 49, 52
+ SE	+	1.02	Zinc finger protein 682	284.48	O95780	1, 2, 3, 5, 7, 9	20, 23	43, 44, 47
+ SE	+	1.02	Zinc finger protein 606	330.32	Q8WXB4	1, 2, 3, 5, 7, 9	20, 23	43, 44, 47
+ SE	+	1.03	Zinc finger protein 721	297.54	B4E159	-	-	-
+ SE	+	1.01	Carcinoembryonic antigen-related cell adhesion molecule 7	260.6	Q14002	-	-	-
+ SE	+	0.99	Transmembrane protein 247	260.56	A6NEH6	-	-	43, 44, 45, 46
+ SE	+	0.98	MTSS1-like protein	316.62	Q765P7	1, 4	20, 21, 26	43, 44, 45, 46

+ SE	+	1.00	Gephyrin (Fragment)	345.48	H0YIY4	-	-	-
+ SE	+	0.98	Cystatin-C	365.38	P01034	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12 ,13, 14, 15, 17, 18, 19	20, 21, 26	43, 44, 45, 46, 47, 48, 49, 52
+ SE	+	1.01	Monocarboxylate transporter 6	226.42	O15375	10	27, 28	43, 46
+ SE	+	1.00	Putative lipocalin 1-like protein 1	301.73	Q5VSP4	-	20	48, 49
+ SE	+	0.94	Zymogen granule protein 16 homolog B	525.96	Q96DA0	6	20	48, 49
+ SE	+	0.99	Alpha-amylase 2B	19911.77	P19961	3, 6	20, 22, 24	48, 49
+ SE	+	0.84	Zinc transporter 6	302.47	Q6NXT4	1, 2, 4, 10	27, 28	43, 44, 45, 46
+ SE	+	0.84	Oxysterol-binding protein-related protein 8	330.69	Q9BZF1	1, 2, 3, 4, 5, 8, 10, 12, 16	20, 25	43, 44, 45, 46, 47
+ SE	+	0.95	Keratin_ type I cytoskeletal 13	763.32	P13646	1, 4, 6, 11, 12, 15	20, 21, 31	43, 44, 45, 47, 48, 49
+ SE	+	0.83	Arginine-glutamic acid dipeptide repeats protein	1158.14	Q9P2R6	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 16	20, 23, 32	43, 44, 47
+ SE	+	0.76	POTE ankyrin domain family member I	1062.35	P0CG38	6	-	48, 49
+ ↓	+	0.61	Negative elongation factor E	545.37	P18615	1, 2, 3, 4, 5, 7, 8, 9, 13	20, 21, 23	43, 44, 46, 47
+ ↓	+	0.55	POTE ankyrin domain family member J	181.39	P0CG39	6	-	48, 49
+ ↓	+	0.87	Basic salivary proline-rich protein 2	2956.72	P02812	-	-	48
+ ↓	+	0.75	Beta-actin-like protein 2	736.45	Q562R1	-	20, 21	43, 44, 45, 48, 49
+ ↓	+	0.47	Bridging integrator 3	240.22	Q9NQY0	1, 2, 4, 6, 11, 12, 16	20, 21	43, 44, 45
+ ↓	+	0.40	Carbonic anhydrase 6	172.46	P23280	1, 3, 4, 6, 10	20, 22, 35	48, 49
+ ↓	+	0.40	Histatin-1	1242.75	P15515	4, 6, 11, 13	20, 21	48
+ ↓	+	0.36	Histatin-3	1349.11	P15516	4, 6, 11, 13	20, 21	48
+ ↓	+	0.70	Immunoglobulin heavy constant alpha 1	2608.5	P01876	1, 2, 3, 4, 5, 6, 8, 10, 13, 16	20, 21	43, 46, 48, 49, 51
+ ↓	+	0.70	Immunoglobulin heavy constant alpha 2	2252.35	P01877	1, 2, 3, 4, 5, 6, 8, 10, 13, 16	20, 21	43, 46, 48, 49, 51
+ ↓	+	0.54	Immunoglobulin J chain	1650.72	P01591	1, 2, 3, 4, 5, 6, 10, 13, 16	20, 21, 23	48, 49

+ ↓	+	0.31	Interferon-induced GTP-binding protein Mx2	349.5	P20592	1, 2, 4, 8, 10, 13	20, 21, 22, 24	43, 44, 45, 46, 47
+ ↓	+	0.87	Keratin_ type II cytoskeletal 4	675.64	P19013	1, 2, 6, 11, 12, 15	20, 21, 31	43, 44, 45, 47, 51
+ ↓	+	0.40	Mitoferrin-1	688.79	Q9NYZ2	10	27, 28	43, 44, 45, 46
+ ↓	+	0.95	Pancreatic alpha-amylase	18247.75	P04746	3, 6, 14	20, 22, 24	48, 49
+ ↓	+	0.72	Polymeric immunoglobulin receptor	592.56	P01833	1, 2, 4, 6, 8, 10, 17	29	43, 44, 45, 46, 48, 49
+ ↓	+	0.14	Protein FAM160B1	265.2	Q5W0V3	-	-	-
+ ↓	+	0.10	RNA-binding protein 25	328.02	P49756	1, 2, 3, 5, 9, 15	20, 21, 23	43, 44, 45, 47
+ ↓	+	0.28	Salivary acidic proline-rich phosphoprotein 1/2	3979.31	P02810	-	20, 21	48, 49
+ ↓	+	0.22	Single Ig IL-1-related receptor (Fragment)	190.19	H0YDR5	-	-	-
+ ↓	+	0.59	Splicing regulatory glutamine/lysine-rich protein 1	251.96	Q8WXA9	1, 2, 3, 5, 9	20, 21, 23	43, 44, 47
+ ↓	+	0.03	Statherin	1767.57	P02808	2, 4, 6, 10, 11, 13, 17	20, 21, 31	48
+ ↓	+	0.76	Submaxillary gland androgen-regulated protein 3B	6879.29	P02814	-	20, 21	48, 49
+ ↓	+	0.15	Zinc finger protein 461	579.79	Q8TAF7	1, 2, 3, 5, 7, 9	20, 23	43, 44, 47

Notes: (+) = presence of protein; (-) = absence of protein, SE = similar expression; (↑) = up-regulated (1-p>0.95); (↓) down-regulated (p<0.05); Ratio A/C = ratio between alcoholism and control groups. [a] **Biological process**: 1 = cellular process; 2 = regulation of biological process; 3 = metabolic process; 4 = response to stimulus; 5 = macromolecule metabolic process; 6 = multicellular organismal process; 7 = biosynthetic process; 8 = cell communication; 9 = nucleobase, nucleoside, nucleotide and nucleic acid metabolic process; 10 = transport; 11 = multicellular organismal development; 12 = cell differentiation; 13 = multi-organism process; 14 = catabolic process; 15 = cell death; 16 = cellular component movement; 17 = secretion; 18 = behavior; 19 = extracellular structure organization. [b] **Molecular function**: 20 = binding; 21 = protein binding; 22 = catalytic activity; 23 = nucleic acid binding; 24 = hydrolase activity; 25 = transferase activity; 26 = enzyme regulator activity; 27 = transporter activity; 28 =ion transmembrane transporter activity; 29 =signal transducer activity; 30 = kinase activity; 31 = structural molecule activity; 32 = oxidoreductase activity; 33 = channel activity; 34 = ligase activity; 35 = lyase activity; 36 = helicase activity; 37 = motor activity; 38 = antioxidant activity; 39 = isomerase activity; 40 = protein transporter activity; 41 = electron carrier activity; 42 = translation regulator activity. [c] **Cellular component**: 43 = cell; 44 = intracellular; 45 = cytoplasm; 46 = membrane; 47 = nucleus; 48 = extracellular region; 49 = extracellular space; 50 = chromosome; 51 = cell surface; 52 = proteinaceous extracellular matrix; 53 =external encapsulating structure.