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Human blood serum proteome changes after 6 hours of sleep deprivation at night

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Abstract

Background: The aim of this study was to discover significantly changed proteins in human blood serum after loss of 6 h sleep at night. Furthermore, to reveal affected biological process- and molecular function categories that might be clinically relevant, by exploring systems biological databases.

Methods: Eight females were recruited by volunteer request. Peripheral venous whole blood was sampled at 04:00 am, after 6 h of sleep and after 6 h of sleep deprivation. We used within-subjects design (all subjects were their own control). Blood serum from each subject was depleted before protein digestion by trypsin and iTRAQ labeling. Labled peptides were analyzed by mass spectrometry (LTQ OritrapVelos Elite) connected to a LC system (Dionex Ultimate NCR-3000RS).

Results: We identified 725 proteins in human blood serum. 34 proteins were significantly differentially expressed after 6 h of sleep deprivation at night. Out of 34 proteins, 14 proteins were up-regulated, and 20 proteins were down-regulated. We emphasized the functionality of the 16 proteins commonly differentiated in all 8 subjects and the relation to pathological conditions. In addition, we discussed Histone H4 (H4) and protein S100-A6/Calcyclin (S10A6) that were upregulated more than 1.5-fold. Finally, we discussed affected biological process- and molecular function categories.

Conclusions: Overall, our study suggest that acute sleep deprivation, at least in females, affects several known biological processes- and molecular function categories and associates to proteins that also are changed under pathological conditions like impaired coagulation, oxidative stress, immune suppression, neurodegenerative related disorder, and cancer. Data are available via ProteomeXchange with identifier PXD021004.

Keywords: Human blood serum, Proteomics, Sleep deprivation, Cellular pathological associations, Stress, and cancer

Background

Sleep deprivation has during the last decade increasingly been explored with discovery-based scientific methodology to test for changes in cellular and molecular mechanisms in different model organisms as well as in humans (O'Callaghan et al., 2019).

The focus in several high-throughput studies has been on gene expression using omic-methodology (Anafi et al., 2013; Archer et al., 2014; Bellesi et al., 2013; Cirelli et al., 2004; Cirelli et al., 2005; Cirelli et al., 2009; Cirelli & Tononi, 2011; Davies et al., 2014; Hinard et al., 2012; Jones et al., 2008; Kim et al., 2014; Mackiewicz et al., 2007; Maret et al., 2007; Miller et al., 2014; Moller-Levet et al., 2013; Pellegrino et al., 2012; Porter et al., 2012; Thompson et al., 2010; Vazquez et al., 2009; Vazquez et al., 2008; Vecsey et al., 2012; Wang et al., 2010; Zimmerman et al., 2006; Aho et al., 2016; Gehrman et al., 2018; Honma et al., 2020; Laing et al., 2017; Nollet et al., 2019; Sengupta et al., 2017; Weljie et al., 2015; Yoon

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et al., 2019), mainly in brain tissue from animal models such as worm (C. elegance) and fruit fly (*D. melanogaster*) (Zimmerman et al., 2006; Naidoo et al., 2012; Bringmann, 2019), mice (Anafi et al., 2013; Bellesi et al., 2013; Mackiewicz et al., 2007; Maret et al., 2007; Thompson et al., 2010; Vecsey et al., 2012; Nollet et al., 2019; Naidoo et al., 2008; Naidoo et al., 2005; Pinotti et al., 2010; Terao et al., 2003; Bruning et al., 2019; Curie et al., 2015; Wisor et al., 2008; Franken & Dijk, 2009; Jones et al., 2015; Massart et al., 2014; Mongrain et al., 2011; Pedersen et al., 2017; Noya et al., 2019) and rats (Cirelli et al., 2009; Kim et al., 2014; Porter et al., 2012; Vazquez et al., 2009; Vazquez et al., 2008; Sengupta et al., 2017) after sleep/wake and sleep deprivation.

Several groundbreaking transcriptomic studies as well as recent metabolomic studies have been published on animals (Weljie et al., 2015; Yoon et al., 2019; Ma et al., 2018; Poirrier et al., 2008; Seo et al., 2008) or humans (Archer et al., 2014; Davies et al., 2014; Moller-Levet et al., 2013; Pellegrino et al., 2012; Aho et al., 2016; Honma et al., 2020; Laing et al., 2017; Laing et al., 2019; Benedict et al., 2014). Moreover, the human circadian metabolome has been explored (Dallmann et al., 2012). However, previous studies in animal models have pointed out that the sleep homeostatic process and the circadian process cannot operate independently and has now also been shown on the protein level and not only at the mRNA level (Curie et al., 2015; Wisor et al., 2008; Franken & Dijk, 2009). Lately, also remote control of the SCN (suprachiasmatic nucleus) circadian clock neurons affecting sleep-wake behavior or states has been effectuated by optogenetics, a powerful method, however, so far only applied in animal models (Jones et al., 2015; Pedersen et al., 2017). Several groups have focused on distinct proteins related to sleep and wake and especially investigated changes in quantity and quality (e.g. phosphorylation) of such proteins after sleep deprivation (Pellegrino et al., 2012; Naidoo et al., 2008; Naidoo et al., 2005; Pinotti et al., 2010; Terao et al., 2003; Bruning et al., 2019; Benedict et al., 2014; Neuner-Jehle et al., 1996; Neuner-Jehle et al., 1995; Seibt et al., 2012).

Nevertheless, high-throughput studies in humans after sleep deprivation are sparse. Hence, there is a need for more studies on the human proteomics after sleep deprivation, particularly using new and more reliable techniques.

Thus, the present study used human blood serum as the most common clinical material available for molecular analysis and with widest diagnostic potential. Among these, proteins frequently have the greatest clinical significance (Anderson, 2010).

The aim of this study was to discover significantly changed proteins in human blood serum after 6-h sleep deprivation at night. Furthermore, to reveal affected cellular and molecular activities that might be clinically relevant, by exploring systems biological databases.

Materials and method

Study participants

Eight healthy female Biomedical laboratory science bachelor-students at Bergen University College (Norway), between 22 and 46 years, were recruited by volunteer request for these experiments. The participants were young, fulltime students and self-declared healthy with no history of neurological or psychiatric disease. All gave their consent to be enrolled in this study. To reduce complexity, we used same sex and within-subject design (Colantonio & Chan, 2005). The rules for the sleep hygiene during the whole research period including control and sleep deprivation night is given in the Supplementary (Table S1). All sleep hygienic data are shown in Table 1.

Sleep monitoring during night

The present study lasted a total of 48 h. The first 24 h included the control night, where the individuals slept from 10:00 pm until next morning at 7:00 am. During the second 24 h, sleep deprivation was performed from

Table 1 Sleep hygiene data

Sociodemographic data	n (number of volunteers), Mean ± SEM
Woman	8
Age, years	27.5 ± 4.3
Height, cm	168.9 ± 1.0
Weight, kg	70.3 ± 5.4
Diet (during study)	same diet for all/regular mealtimes
Physical activities (hours /week)	3.5 ± 0.5
Smoking	0/8
Contraceptives	4/8
Medication (other than contraceptives)	0/8

10:00 pm until 04:00 am in the morning. Venous blood was carefully sampled at 04:00 am from all participants, and thus during the control night they were slightly disturbed but not fully awake (according to the sleep registration). All samples were collected with the subjects in the supine position and controls at least minimum 15 min before sampling (Miller et al., 1992). All subjects were their own control. Blood samples were allowed to coagulate for 30 min after sampling and then centrifuged for 10 min at 1500 rpm within 30 min. Blood serum was then collected and stored at − 80 °C (Adkins et al., 2002). During the 6 h of sleep deprivation, the subjects maintained the awake status by reading or watching TV (not activating programs). Lights went off at 22:00 both nights. Sleep was tracked simultaneously with an Oura ring and Somnofy (Toften et al., 2020). Briefly, the Oura ring detects heart rate, respiratory rate, body temperature and movements, while Somnofy is a contactless sleep and breath monitor it also detects environmental factors (temperature, humidity, light, and sound). Both are validated against the gold standard polysomnography and found to strongly correlate (Toften et al., 2020).

Depletion of high-abundant proteins from human serum samples

To identify protein changed after sleep deprivation, mass spectrometry and system biological based data analysis was used. 20 μl of blood serum from each subject was depleted using a human Multiple Affinity Removal System (MARS Hu − 14) 4.6 mm × 50 mm LC column (Agilent Technologies), as previously described, utilizing the Dionex 3000-series LC system (Kroksveen et al., 2013).

Peptide extraction and iTRAQ labeling

The entire depleted protein sample was denatured using 6 M urea and dithitreoitol, cysteine blocked using iodoacetamide, and trypsin digested (1:20, trypsin:protein, w/w) according to Berle et al. (Berle et al., 2013) Trypsin was purchased from Promega (Madison, Wisconsin, USA). iTRAQ labeled (4-plex) was combined according to the protocol using the chemicals provided (AB Sciex, USA). N-octyl-β-D-glycopyranoside (NOG), acetonitrile (ACN), formic acid (FA), ammonium formate and water were purchased from Sigma-Aldrich.

Mix-mode fractionation

iTRAQ labeled peptides were fractionated into 50 fractions using a mix-mode chromatography utilizing a SielcPromix column (MP − 10.250.0530, 1.0 × 250 mm, 5 μm, 300 Å, Sielc Technologies, Prospect Heights, Illinois), and an Agilent 1260 series LC system (Agilent Technologies, Palo Alto, CA) as previously described (Berle et al., 2013). The fractions from the 10 first minutes of the gradient were discarded.

LC-MS/MS analyses

Fifty mix mode fractions from each iTRAQ experiment were analyzed on an LTQ-OrbitrapVelos Elite connected to a Dionex Ultimate NCR-3000RS LC system (Thermo Fisher Scientific). The fractions were dissolved in 1% FA and trapped on the pre-column (Dionex, AcclaimPepMap 100, 2 cm × 75 μm i.d, 3 μm C18 beads) in buffer A (2% ACN, 0,1% FA), at a flowrate of 5 μl/min for 5 min before separation by reverse phase chromatography (Dionex, Acclaim PepMap 100, 15 cm × 75 μm i.d., 3 μm C18 beads) at a flow of 280 nL/min. The mix mode fractions were run on three slightly different nano LC gradients. The first fifteen fractions were run on a LC gradient consisted of a gradient starting at 5% buffer B (90%ACN, 0.1% FA) ramping to 12% buffer B over 55 min (5–60 min). Further, the gradient was ramped to 30% buffer B in 30 min (60-90 min), increased to 90% B in 10 min (90–100 min), held for 5 min (100–105 min) followed by ramping to 5% buffer B for 3 min (105–108) and equilibration of the column in 12 min (108–120). Fraction 16–35 were separated on the following LC gradient; 0–5.0 min 5% buffer B, 5.0–5.5 min 8% buffer B, 5.5–60 min 20% buffer, 60–90 min 35% buffer B. The last fractions (36–50) were separated on the following gradient: 0–5.0 min 5% buffer B, 5.0–5.5 min 8% buffer B, 5.5–90 min 40% buffer. The last part of the nano LC gradient is similar for all three gradients. Full scan MS spectra were acquired in the Orbitrap-MS with resolution R = 120,000 at m/z 400. The 10 most intense eluting peptides above 1000 counts and charge states 2 or higher, were sequentially isolated in the linear ion trap. Fragmentation in the Higher-Energy Collision Dissociation (HCD) cell was performed with a normalized collision energy of 40%, and activation time of 0.1 milliseconds. Fragments were detected in the Orbitrap-MS at a resolution of 15,000.

Pre-analysis of the identified proteins before database's analysis

iTRAQ results were analyzed to verify the corresponding proteins. Initially 807 proteins were detected from raw data. Some proteins were not considered in our experiment due to the following reasons: resence of different peptides (62 discarded), reverse peptides (2 discarded), peptides without signals (2 discarded) and proteins removed by the MARS (Multi Affinity Removal System) column (16 discarded). Finally, 725 proteins were considered for our blood serum proteome experiment.

The systems biological database WebGestalt recommends using Entrezgene ID as molecule identifier for the analysis. Therefore, 725 Swissprot proteins IDs were

converted to Entrezgene ID by using online software BIOMART. MetaCore and GeneCards have been used to complete the conversions.

Gene ontology and enrichment analysis

A systems biological data analysis was performed by two different databases WebGestalt and MetaCore.

In the systems biological database WebGestalt, we selected the Gene Ontology (GO) slim classification and Gene Ontology Enrichment Analysis (GO-EA) to explore our data among three domains: Biological process, Molecular function, and Cellular component.

The GO slim classification corresponds to an overview analysis containing a subset of the terms or categories in the whole GO. On the other hand, the GO enrichment analysis (GO-EA) represents the up and down- regulated GO terms or categories using the annotation for this set of genes reflecting our changed proteins in a certain category (Zhang et al., 2005). The goal of these analysis was to identify the most affected biological processes and molecular functions after 6 h of sleep deprivation.

In the WebGestalt database (Zhang et al., 2005), first, the affected biological process and molecular function categories were identified based on our changed proteins, by Gene Ontology Slim classification. Further, the most affected biological process categories by Gene Ontology enrichment analyses were successively classified after mostly affected versus less affected biological process categories after 6 h of sleep deprivation.

In the GO-EA we identify the top 10 enriched biological process using the default settings of WebGestalt.

By the other system biological data base MetaCore we only carried out the Enrichment Analysis, that include several ontologies due to the enrichment of the uploaded data. However, we focused in the GO processes, referring to the GO Biological process, and compared the results from the GO Biological Process Enrichment Analysis, from both databases.

Protein accession numbers were converted to official gene symbols using the Perseus software (Tyanova et al., 2016). The data were sorted based on four valid values in at least one group and imputed using default settings in Perseus. The data was transferred to J-express where gene set enrichment analysis (GSEA) was conducted (Stavrum et al., 2008). Gene signatures were downloaded from the MSig database (www.broadinstitute.org/gsea) according to Subramanian et al. (Subramanian et al., 2005). Briefly explained, genes were ranked based on their expression in each sample and then incorporated in each respective gene expression signature. A normalized enrichment score (NES) was the calculated for the gene signature before and after sleep depression together with a *p*-value and a permutation-based fold discovery rate (FDR).

Data analysis and statistics

The iTRAQ MS/MS data was searched against the Uniprot human database (UniProt, 2019) using SearchGUI (v1.14.4) (Vaudel et al., 2011), utilizing search engines OMSSA (Geer et al., 2004) and X!Tandem (Fenyo & Beavis, 2003). The search parameters were precursor mass tolerance 10 ppm, product mass tolerance 0.6 Da and maximum two missed cleavages. Fixed modifications used were iTRAQ (K and n-term) and methylthio (C) and variable modifications used were oxidation of methionine and iTRAQ (Y). The SearchGUI output was post-processed in PeptideShaker [http://peptide-shaker.googlecode.com] using an FDR-level of 1%, and the results further processed by an in-house script to extract the intensities of the iTRAQ reporters (114, 115, 116, 117). The intensities were normalized to 1 and converted to log2 values. Finally, the quantitative data from the subjects was compared by a paired two-tailed t-test. Furthermore, to explore interactions between the 34 statistically differentially expressed proteins we used the downloadable STRING app (Szklarczyk et al., 2019) in Cytoscape (version 3.8.2) (Shannon et al., 2003).

Table 2 Physiological and sleep data

	OUR-RING		SOMNOFY	
	Control	Sleep deprivation	Control	Sleep deprivation
Physiological data				
Heart rate (min)	62 ± 7	59 ± 10	58 ± 10	53 ± 6
Respiratory rate (min)	15 ± 0,4	15 ± 1,4	15 ± 1,0	17 ± 0,9
Sleep data				
Total sleep time (hours)	7.42 ± 0.43	3.24 ± 0.35*	7.07 ± 0.26	3.37 ± 0.19*
REM sleep (%)	14.3 ± 2.1	7.1 ± 2.6 *	25.3 ± 1.3 °	22.1 ± 2.9°
Light sleep (%)	66.1 ± 3.4	49.8 ± 9.2	54.3 ± 2.5	35.7 ± 5.0
Deep sleep (%)	19.5 ± 2.9	43.0 ± 8.0*	20.4 ± 3.2	42.3 ± 5.1*

°$p < 0.05$ vs Oura-ring data.
*$P < 0.05$ vs control

Results

All physiological and sleep data is shown in Table 2.

Identified proteins in human serum using mass spectrometry

We identified 807 proteins in human blood serum by iTRAQ mass spectrometry analysis, either up- or down-regulated after 6 h of sleep deprivation at night in a within-subject design pilot study. After several manual filtering criteria and adjustments, as described in materials and method, we report 725 of the 807 proteins. Among the 725 proteins, 377 proteins were upregulated, and 348 proteins downregulated after 6 h of sleep deprivation at night.

Out of the 725 identified proteins, 34 proteins were significantly changed after 6 h of sleep deprivation at night (p-value < 0.05). Out of 34 proteins, 14 proteins were upregulated and 20 proteins were downregulated (Table 3). The 16 differentially expressed proteins found in all 8 subjects, are shown as bold and red in Table 3.

A protein-protein interaction network created in Cytoscape using the String app for the 34 proteins significantly changed shows that 15 of the proteins are connected to either one or more proteins based on co-expression, database search or experiments (Fig. 1).

Biological process and molecular function

We also compared different GO classifications based on biological process and molecular function of the following three groups of proteins (Tables 4 and 5); the total identified proteins (725), the changed proteins after 6 h of sleep deprivation (34) and changed proteins common in all 8 subjects (16). More importantly and quite remarkable, approximately half of the significantly changed proteins after sleep deprivation from all groups, were involved in the same four biological process categories: biological regulation, response to stimulus, metabolic process, and multicellular organismal process.

Similarly, we compared the same three groups of proteins regarding the molecular function categories by Gene Ontology slim analysis. Two of the categories of molecular function; Protein Binding and Ion binding categories included approximately the half of the proteins in each of these two different groups of proteins (Table 5).

Biological process categories compared by two different databases

We also compared top scored biological process ontology categories by Gene Ontology enrichment analyses of the 16 proteins similarly changed in all eight subjects after 6 h sleep deprivation at night in two different databases, MetaCore and WebGestalt. Most of the enriched biological process categories relates to the following: 1. Protein metabolic process, 2. Immune system and complement activation, 3. Immune system and humoral response and 4. Blood coagulation. Therefore, we presented four groups of the biological processes together in Table 5. Four proteins Plasma protease C1 inhibitor (IC1), Complement C1r subcomponent (C1R), ADP-ribosyl cyclase 2 (BST1) and Mannan-binding lectin serine protease 1 (MASP1) were classified into the biological process category for Immune system and humoral response, where three of them, IC1, C1R, and MASP1 were the same proteins as classified into two other biological process categories; The protein metabolic process and Immune system and Complement activation. Also, the proteins IC1 and MASP1 were present in the three groups out of four significantly affected biological process categories by Gene Ontology enrichment analyses (Table 6).

$p < 0.05$).

We have also analyzed our increased and/or decreased identified proteins in human serum after 6 h sleep deprivation at night with Ingenuity as indicated in Table 7. This showis association to several common types of cancer. Half of the 34 and the 16 changed proteins after sleep deprivation have been associated with changed expression of the same respective genes associated with urogenital and urological cancers, respectively. Also, more than half of our significantly changed proteins (34) after 6 h sleep deprivation at night is associated with their respective genes in liver-related cancer. Finally, seven proteins related to similar gene-expression changes in breast cancer were also seen, including one of the two mostly changed proteins in our dataset in relation to fold-change, namely S100A.

As shown in Table 8, changes in several of our proteins are comparable to findings by others after sleep loss conditions in tissues and/or body fluids.

Our changed proteins after sleep deprivation are assoiated to breast cancer mRNA signatures and cell junction organization in Gene Set Enrichmnet Analysis (GSEA) as shown in Fig. 2 and Table 9.

Cellular response to stress ($P \leq 0.01$), Cell junction organization ($P \leq 0.01$), Vantveer breast cancer ($P \leq 0.01$) and SMID breast cancer basal up ($P \leq 0.01$).

Discussion

In the current study, 725 proteins were identified in human (female) blood serum. 377 proteins were up-regulated and 348 proteins down-regulated after 6 h of sleep deprivation at night. Out of the 725 identified proteins, 34 proteins were significantly changed after 6 h of sleep deprivation at night. Out of 34 proteins, 14 proteins were up-regulated, and 20 proteins were down-regulated. However, the most important finding is that 16 out of the 34 proteins were found

Table 3 Proteins in human serum after 6 h of sleep deprivation identified by iTRAQ analysis

Swissprot ID	Protein Short Name	Protein Name	*P*-value	Average foldchange
Up-regulated proteins (14)				
Q9HBW9	ELTD1	EGF latrophilin and seven transmembrane domain-containing protein 1	0,0002	1064
P62805	H4	Histone H4	0,0067	2291
P07951	TPM2	Tropomyosin beta chain	0,0082	1153
P0CG38	POTEI	POTE ankyrin domain family member I	0,0088	1035
P23141	EST1	Liver carboxylesterase 1	0,0102	1075
Q6ZMR3	LDH6A	L-lactate dehydrogenase A-like 6A	0,0143	1,11
P06703	S10A6	Protein S100-A6	0,0145	1525
P01880	IGHD	Ig delta chain C region	0,0247	1184
Q86T29	ZN605	Zinc finger protein 605	0,0438	1059
A5A3E0	**POTEF**	**POTE ankyrin domain family member F**	**0,0441**	**1036**
P13716	HEM2	Delta-aminolevulinic acid dehydratase	0,0454	1115
P33151	**CADH5**	**Cadherin-5**	**0,0454**	**1074**
P02144	MYG	Myoglobin	0,0476	1094
P55285	**CADH6**	**Cadherin-6**	**0,0499**	**1036**
Down-regulated proteins (20)				
P05155	**IC1**	**Plasma protease C1 inhibitor**	**0,0023**	**– 1129**
P00390	GSHR	Glutathione reductase, mitochondrial	0,0029	– 1068
P23470	**PTPRG**	**Receptor-type tyrosine-protein phosphatase gamma**	**0,0036**	**– 1058**
O75022	LIRB3	Leukocyte immunoglobulin-like receptor subfamily B member 3	0,0048	– 1101
P00736	**C1R**	**Complement C1r subcomponent**	**0,006**	**– 1047**
Q8TDY8	IGDC4	Immunoglobulin superfamily DCC subclass member 4	0,0134	– 1065
Q10588	**BST1**	**ADP-ribosyl cyclase 2**	**0,0169**	**– 1066**
P08709	**FA7**	**Coagulation factor VII**	**0,0186**	**–1,05**
P07942	**LAMB1**	**Laminin subunit beta – 1**	**0,0188**	**– 1022**
P08294	**SODE**	**Extracellular superoxide dismutase [Cu-Zn]**	**0,0199**	**–1,05**
Q9UHG3	**PCYOX**	**Prenylcysteine oxidase 1**	**0,0208**	**– 1067**
O75326	SEM7A	Semaphorin-7A	0,0212	– 1057
P35542	SAA4	Blood serum amyloid A-4 protein	0,0223	–1058
Q86VB7	**C163A**	**Scavenger receptor cysteine-rich type 1 protein M130**	**0,0357**	**– 1063**
P23142	**FBLN1**	**Fibulin – 1**	**0,036**	**– 1038**
P48740	**MASP1**	**Mannan-binding lectin serine protease 1**	**0,0387**	**– 1073**
Q8N3C0	ASCC3	Activating signal cointegrator 1 complex subunit 3	0,0414	-1105
P24387	CRHBP	Corticotropin-releasing factor-binding protein	0,0417	-1066
P58215	**LOXL3**	**Lysyl oxidase homolog 3**	**0,0457**	**-1084**
O75882	**ATRN**	**Attractin**	**0,0469**	**-1039**

All changed proteins (34) in human serum, up- and down- regulated after 6 h of sleep deprivation at night in a within subject design experiment controlled for circadian time. The 16 differentially expressed proteins found in all 8 subjects are shown as bold and enlarged. $p < 0.05$.

similarly changed in all 8 subjects and their biological associations/functions are as follow: impaired coagulation, immune suppression, oxidative stress, neurodegenerative diseases, and cancer in at least females.

Below we will discuss first, each of the single protein and their functions before identifying the relations between the proteins and their biological process and molecular functions.

Upregulated proteins (3) in all subjects

POTE (Prostate, Ovary, Testes and Embryo) ankyrin domain family member F (POTEF) was found to be

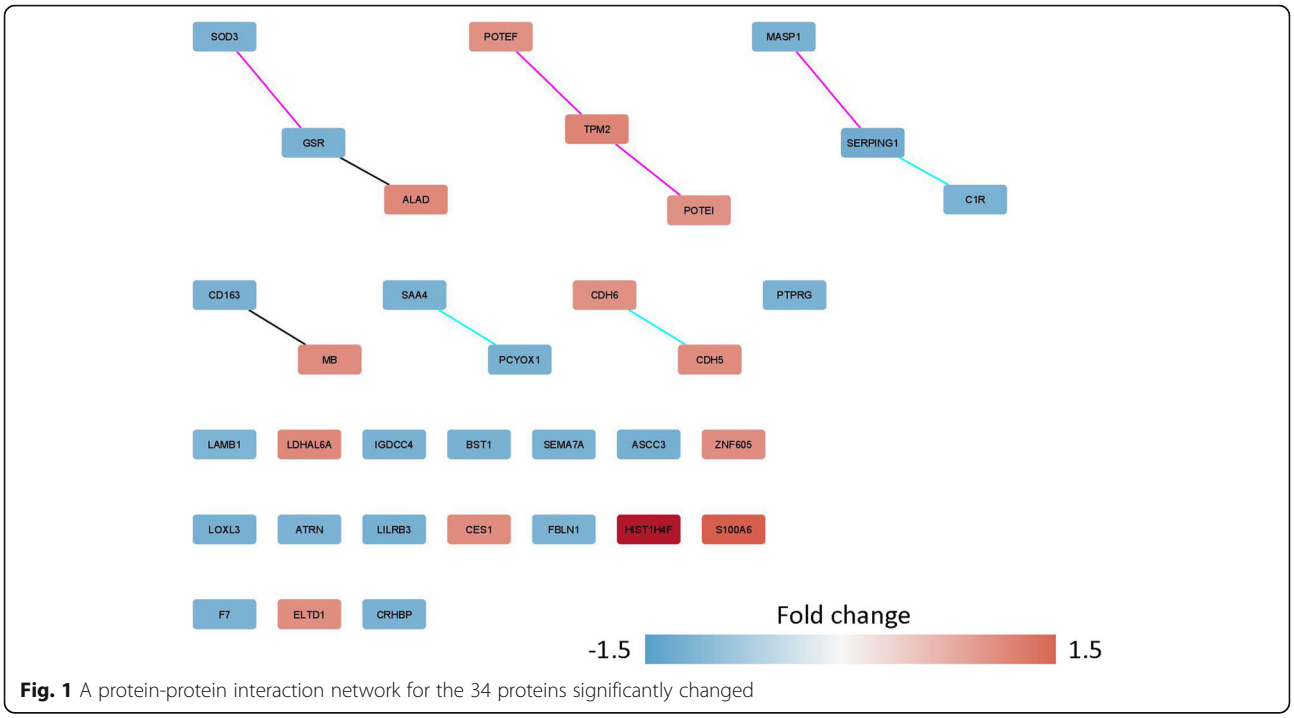

Fig. 1 A protein-protein interaction network for the 34 proteins significantly changed

significantly upregulated in the present study. POTE expression has been reported in several forms of human cancer such as prostate, colon, lung, ovary, pancreas, and breast but only in a limited number of normal organs, including prostate, ovary, testis, and placenta (Vekariya et al., 2019). Due to their tumor-specific expression, POTEs are potential oncogenes, therapeutic targets, and biomarkers for these malignancies (Maggiolini et al., 2020). Thus, 6 h of sleep deprivation has elevated this tumor potentiating protein.

We also found a significant upregulation of **Cadherin-5 and 6 (CADH5 and CADH6)**. Cadherins are calcium-dependent cell adhesion proteins. They function as classical cadherins by imparting to cells the ability to adhere in a hemophilic manner. The CADH5 protein plays a role in endothelial adherence, junction, and assembly and has shown to be associated with vascular disease (Lampugnani et al., 2018). Thompson et al. (Thompson et al., 2010) also found CADH5 upregulated after 6 h of sleep deprivation, however, CADH6 was downregulated in entorhinal cortex in mice at the transcription level.

Furthermore, two human blood serum proteins, among all the changed proteins (34) after 6 h of sleep deprivation at night, were up-regulated more than 1.5-fold; Histone H4 (H4) and protein S100-A6/Calcyclin (S10A6).

Histones are a family of small, positively charged proteins termed H1, H2A, H2B, H3, and H4 (Zlatanova et al., 1998). Change in the level of H4, one of the four DNA-packing proteins, might affect the whole transcriptional machinery, disturbing gene transcription and thereby affecting translation e.g. the production of proteins generally and lead to change in cellular processes and networks as we have observed, reflected in Tables 2, 3, 4 and 5. Histone H4 as we found to be upregulated after acute sleep deprivation in human blood serum, has been reported downregulated in rat cerebral cortex (Cirelli et al., 2009). However, this might be explained by compartmentalization and transport of this protein from cytoplasm to nucleus in the cells (Djordjevic et al., 2009), thereby leaving less of the protein to diffuse extracellularly and hence to the bodily fluids as serum. However, and in line with our finding, most other studies report upregulation, however non-significant, of several variants or subtypes of Histone transcripts in different part of brain cortex in mice (Mackiewicz et al., 2007; Thompson et al., 2010). Also, Histone acetylation weakens histone-DNA interactions resulting in a relaxed chromatin conformation, facilitating recruitment of transcriptional machinery, and are also affected after sleep deprivation (Gaine et al., 2018). Also, pointed out in the same article, affected histones, especially H3 and H4, was related to the circadian clock disruption after sleep deprivation and also its effect on cAMP response element-binding protein (CREB) and brain derived neurotrophic factor (BDNF), important signaling and memory associated proteins (Gaine et al., 2018; Basheer et al., 2005).

We also found an increase in **S100-A6, also named Calcyclin**, over 1.5-fold in human blood serum after one

Table 4 Biological process categories by Gene Ontology (GO) slim classification of the total identified proteins (725), the changed proteins (34) after 6 h of sleep deprivation (SD) and changed proteins common in all 8 subjects (16) after 6 h of SD

Biological process categories of GO slim classification	Total identified proteins (725*)(*n* = 8)		Changed proteins after 6 h of sleep deprivation (34)(n = 8)			Changed proteins common in all 8 subjects (16)(n = 8)		
	Number of proteins	Percentage	Number of proteins in each biological process category	Protein Short Name	Percentage	Number of proteins in each biological process category	Protein Short Name	Percentage
Biological Regulation	432	61,63%	19	↓ASCC3, ↓ATRN, ↓C1R,↑CADH5, ↓CRHBP, ↑ELTD1, ↓FA7, ↓GSHR, ↓IC1, ↓LAMB1, ↓LIRB3, ↓LOXL3, ↓MASP1, ↑MYG, ↓PTPRG, ↑S10A6, ↓SEM7A, ↑TPM2, ↑ZN605	55,88%	9	↓ATRN, ↓C1R, ↑CADH5, ↓FA7, ↓IC1, ↓LAMB1, ↓LOXL3, ↓MASP1, ↓PTPRG	56,25%
Response to Stimulus	420	59,91%	20	↓ASCC3, ↓ATRN, ↓BST1, ↓C163A, ↓C1R, ↓CRHBP, ↑ELTD1, ↑EST1, ↓FA7, ↓IC1, ↑IGHD, ↓LAMB1, ↓LIRB3, ↓MASP1, ↑MYG, ↓PTPRG, ↑S10A6, ↓SAA4, ↓SEM7A, ↓SODE	58,82%	10	↓ATRN, ↓BST1, ↓C163A, ↓C1R, ↓FA7, ↓IC1, ↓LAMB1, ↓MASP1, ↓PTPRG, ↓SODE	62,50%
Metabolic Process	399	56,92%	16	↓ASCC3, ↓C1R, ↓CRHBP, ↑EST1, ↓FA7, ↓GSHR, ↑HEM2, ↓IC1, ↑LDH6A, ↓LOXL3, ↓MASP1, ↓PCYOX, ↓PTPRG, ↓SODE, ↑TPM2, ↑ZN605	47,06%	8	↓C1R, ↓FA7, ↓IC1, ↓LOXL3, ↓MASP1, ↓PCYOX, ↓PTPRG, ↓SODE	50,00%
Multicellular organismal process	370	52,78%	15	↓ATRN, ↓BST1, ↑CADH5, ↓CRHBP, ↓FA7, ↓FBLN1, ↓GSHR, ↓IC1, ↓LAMB1, ↓LIRB3, ↓LOXL3, ↑MYG, ↑S10A6, ↓SEM7A, ↑TPM2	44,12%	8	↓ATRN, ↓BST1, ↑CADH5, ↓FA7, ↓FBLN1, ↓IC1, ↓LAMB1, ↓LOXL3	50,00%
Developmental process	290	41,37%	10	↓BST1, ↑CADH5, ↓FA7, ↓FBLN1, ↓LAMB1, ↓LIRB3, ↓LOXL3, ↑MYG, ↑S10A6, ↓SEM7A	29,41%	6	↓BST1, ↑CADH5, ↓FA7, ↓FBLN, ↓LAMB1, ↓LOXL3	37,50%
Localization	234	33,38%	4	↓CRHBP, ↓FA7, ↓IC1, ↓LAMB1	11,76%	3	↓FA7, ↓IC1, ↓LAMB1	18,75%
Cellular Component Organization	220	31,38%	8	↑CADH5, ↑CADH6, ↓FBLN1, ↑HEM2, ↓LAMB1, ↓LOXL3, ↑S10A6, ↓SEM7A	23,53%	5	↑CADH5, ↑CADH6, ↓FBLN1, ↓LAMB1, ↓LOXL3	31,25%
Cell Communication	214	30,53%	7	↓CRHBP, ↑ELTD1, ↓FA7, ↓LIRB3, ↓PTPRG, ↑S10A6, ↓SEM7A	20,59%	2	↓FA7, ↓PTPRG	12,50%
Cell Proliferation	108	15,41%	4	↓ASCC3, ↑CADH5, ↓LAMB1, ↑S10A6	11,76%	2	CADH5, LAMB1	12,50%
Death	100	14,27%	1	↓FA7	2,94%	1	↓FA7	6,25%
Multi-Organism Process	80	11,41%	3	↓CRHBP, ↓FBLN1, ↓LAMB1	8,82%	2	↓FBLN1, ↓LAMB1	12,50%
Reproduction	78	11,13%	4	↓CRHBP, ↓FBLN1, ↓GSHR, ↓LAMB1	11,76%	2	↓FBLN1, ↓LAMB1	12,50%
Growth	68	9,70%	2	↓ATRN, ↓SEM7A	5,88%	1	↓ATRN	6,25%
Unclassified	97	13,84%	4	↑H4, ↓IGDC4, ↑POTEF, ↑POTEI	11,76%	1	↑POTEF	6,25%

Comparison of biological process categories based on GO slim classifications of our 3 different groups of proteins (725, 34 and 16) in the system biological database WebGestalt. The underlined proteins were found in both groups of proteins (34 and 16) after 6 h of sleep deprivation. The ↑ and ↓ represent up- and down-regulated proteins, respectively. For full name of the protein, see Table 3. *Entrezgene identifier is recommended to use in WebGestalt, therefore we converted the 725 Swissprot IDs ending up with 701 Entrezgene IDs, used in this analysis. $p < 0.05$).

Table 5 Molecular function categories by Gene Ontology (GO) slim classification of the total identified proteins (725), the changed proteins (34) after 6 h of sleep deprivation (SD) and changed proteins common in all 8 subjects (16) after 6 h of SD

Molecular function categories of GO slim classification	Total identified proteins (725*)		Changed proteins after 6 h of sleep deprivation (34)			Changed proteins common in all 8 subjects (16)		
	Number of proteins	Percentage	Number of proteins in each molecular function category	Protein Short Name	Percentage	Number of proteins in each molecular function category	Protein Short Name	Percentage
Protein Binding	363	51,78%	18	↓ASCC3, ↓C163A, ↑CADH5, ↓CRHBP, ↑ELTD1, ↓FA7, ↓GSHR, ↑HEM2, ↓IC1, ↓LAMB1, ↓LIRB3, ↓LOXL3, ↓MASP1, ↓PTPRG, ↑S10A6, ↓SEM7A, ↓SODE, ↑TPM2	52,94%	9	↓C163A, ↑CADH5, ↓FA7, ↓IC1, ↓LAMB1, ↓LOXL3, ↓MASP1, ↓PTPRG, ↓SODE	56,25%
Ion Binding	275	39,23%	17	↓ASCC3, ↓C1R, ↑CADH5, ↑CADH6, ↑ELTD1, ↓FA7, ↓FBLN1, ↓GSHR, ↑HEM2 ↓LOXL3, ↓MASP1, ↑MYG, ↑POTEF, ↑POTEI, ↑S10A6, ↓SODE, ↑ZN605	50,00%	9	↓C1R, ↑CADH5, ↑CADH6, ↓FA7, ↓FBLN1, ↓LOXL3, ↓MASP1, ↑POTEF, ↓SODE	56,25%
Hydrolase Activity	139	19,83%	8	↓ASCC3, ↓BST1, ↓C1R, ↑EST1, ↓FA7, ↓MASP1, ↓PCYOX, ↓PTPRG	23,53%	6	↓BST1, ↓ C1R, ↓FA7, ↓MASP1, ↓PCYOX, ↓PTPRG	37,50%
Enzyme Regulator Activity	72	10,27%	2	↓FBLN1, ↓IC1	5,88%	2	↓FBLN1, ↓IC1	12,50%
Nucleotide Binding	72	10,27%	6	↓ASCC3, ↓BST1, ↓GSHR, ↑LDH6, ↑POTEF, ↑POTEI	17,65%	2	↓BST1, ↑POTEF	12,50%
Structural Molecule Activity	66	9,42%	3	↓FBLN1, ↓LAMB1, ↑TPM2	8,82%	2	↓FBLN1, ↓LAMB1	12,50%
Molecular transducer Activity	65	9,27%	3	↑ELTD1, ↓LIRB3, ↓PTPRG	8,82%	1	↓PTPRG	6,25%
Transferase Activity	56	7,99%	0		0%	0		0%
Lipid Binding	43	6,13%	1	↓LAMB1	2,94%	1	↓LAMB1	6,25%
Carbohydrate Binding	41	5,85%	1	↓ATRN	2,94%	1	↓ATRN	6,25%
Nucleic Acid Binding	39	5,56%	2	↓ASCC3, ↑ZN605	5,88%	0		0%
Transporter Activity	34	4,85%	3	↑MYG, ↓PCYOX, ↑S10A6	8,82%	1	↑PCYOX	6,25%
Antioxidant Activity	16	2,28%	2	↓GSHR, ↓SODE	5,88%	1	↓SODE	6,25%
Oxygen Binding	8	1,14%	1	↑MYG	2,94%	0		0%
Electron Carrier Activity	4	0,57%	1	↓GSHR	2,94%	0		0%
Molecular Adaptor Activity	2	0,29%	0		0%	0		0%
Chromatin binding	3	0,43%	0		0%	0		0%
Unclassified	104	14,84%	4	↑H4, ↓IGDC4, ↑IGHD, ↓SAA4	11,76%	0		0%

Comparison of molecular function categories based on GO slim classifications of our 3 different groups of proteins (725, 34 and 16) in the system biological database WebGestalt. The underlined proteins were found in both groups of proteins (34 and 16) after 6 h of sleep deprivation. The ↑ and ↓ represent up- and down-regulated proteins, respectively. For full name of the protein, see Table 3. *Entrezgene identifier is recommended to use in WebGestalt, therefore we converted the 725 Swissprot IDs ending up with 701 Entrezgene IDs, used in this analysis. $p < 0.05$).

night of sleep deprivation for 6 h. Downregulation of S100 A6 transcripts was found in different part of brain cortex in mice after sleep deprivation in two earlier studies by Thompson and Mackiewicz (Mackiewicz et al., 2007; Thompson et al., 2010). Porter et al. (Porter et al., 2012) found S100-A6 in their transcriptomic studies of mice and rat brain cortex or entorhinal cortex and in the hippocampus to be downregulated after 3 to 24 h of sleep deprivation (Mackiewicz et al., 2007; Porter et al., 2012; Thompson et al., 2010). On the contrary, and in

Table 6 Biological process categories by Gene Ontology (GO) enrichment analysis of changed proteins common in all 8 subjects (16) compared in two different databases, MetaCore (MC) and WebGestalt (WG)

Order of biological process		Biological process categories of GO enrichment analysis	Proteins short name involved among the 16	MetaCore Database FDR	WebGestalt Database adjP
MC	WG	**Protein metabolic process**			
1	1	Protein activation cascade	IC1, C1R, FA7, MASP1	1,35E-03	0,0002
9	9	Regulation of protein activation cascade	IC1, MASP1	1,93E-02	0,0163
3	4	Negative regulation of protein activation cascade	IC1, MASP1	3,68E-03	0,0016
18	8	Negative regulation of protein processing	IC1, MASP1	(5,79E-02)*	0,0105
		Immune system and complement activation			
6	7	Complement activation	IC1, C1R, MASP1	5,79E-03	0,0057
8	10	Regulation of complement activation	IC1, MASP1	1,82E-02	0,0163
2	3	Negative regulation of complement activation	IC1, MASP1	3,68E-03	0,0016
11	6	Complement activation, lectin pathway	IC1, MASP1	(5,15E-02)*	0,0045
		Immune system and humoral response			
4	2	Humoral immune response	IC1, C1R, BST1, MASP1	3,68E-03	0,001
10	**	Regulation of humoral immune response	IC1, MASP1	4,36E-02	not present in top 10
5	5	Negative regulation of humoral immune response	IC1, MASP1	3,68E-03	0,0024
		Blood coagulation			
7	**	Blood coagulation, fibrin clot formation	IC1, FA7	1,09E-02	not present in top 10

Each database use different statistical significance tests. *FDR* False Discovery Rate; *adjP p*-value adjusted by the multiple test adjustment. For full name of the protein, see Table 3. *FDR > 0.05. ** WebGestalt database does not give classification more than/above top 10 biological process categories.

line with our findings, studies in human bodily fluid such as urine and blood serum, the protein (and not only transcript) S100-A6 has been shown to increase after 8 h of sleep deprivation (Benedict et al., 2014). In urine, the protein S100-A6 was also found to be increased after obstructive sleep apnea (OSA) and thereby in part sleep deprivation (Becker et al., 2014). The suggested effects of S100 A6 in Filipeck et al. (Filipek et al., 2008) and Naidoo et al. (Naidoo et al., 2008) fit well with possible involvement of S100A6 in various cellular processes as cell differentiation and ubiquitination indicating that this small Ca2þ-binding protein plays an important role in cell homeostasis.

Downregulated proteins in all subjects

Plasma protease C1 inhibitor (IC1) also named Serpin family member 1 (SERPING1) was downregulated in the present study. This corresponds to other studies after sleep deprivation (Becker et al., 2014; Liu et al., 2009). This protein has also been shown in a novel proteomic study in idiopathic REM sleep behavior disorder to be differentially expressed (Mondello et al., 2018). It has been indicated to play a crucial role in regulating important physiological functions including complement activation, blood coagulation (FXIIa inhibitor), fibrinolysis and the generation of kinins. This decrease of IC1 could thereby possibly lead to increased coagulation as seen by others after just one night of sleep deprivation and compromise the immune system (Liu et al., 2009). Thus, sleep deprivation may worsen systemic inflammation and hypercoagulable states, which are known to be involved in the pathogenesis of diseases such as cerebrovascular or cardiovascular disease (Liu et al., 2009).

Receptor-type tyrosine-protein phosphatase gamma or **Protein Tyrosine Phosphatase Receptor Type G (PTPRG)** was downregulated after 6 h of sleep deprivation and this corresponds to a downregulated transcript in rat brain hippocampal tissue after 24 and 72 h of sleep deprivation (Porter et al., 2012). PTPRG is a signaling molecule regarding cellular processes including differentiation, mitotic cycle, and oncogenic transformation (Cheung et al., 2015). This protein has been shown in a novel proteomic study in idiopathic REM sleep disorder to be differentially expressed (Mondello et al., 2018). A reduction in PTPRG might have a function in negative regulation of neuronal projection (Baker et al., 2000). The protein tyrosine phosphatases PTPRZ and PTPRG binds to distinct members of the contactin family of neural recognition molecules. Overall, these findings implicate PTPRG, PTPRZ and CNTNs as a group of receptors and ligands involved in the manifold recognition events that underlie the construction of neural networks are compromised after sleep deprivation (Bouyain & Watkins, 2010; Owen et al., 2021). Since

Table 7 Genes representing our significantly changed proteins (34) after 6 h of sleep deprivation enriched in different cancers by Ingenuity system biological database

Cancer type	Number of total genes involved in this cancer from our 34	Genes common in: ALL 4 cancer types urogenital, urological, breast, liver (3)	Genes common in: 3 cancer types: urogenital, breast and liver (2)	Genes common in: 3 cancer types: urogenital, urological and liver (5)	Genes common in: 2 cancer types: breast and liver (1)	Genes common in: 2 cancer types urogenital and liver (4)	Genes common in: 1 cancer: breast (1)	Genes common in: 1 cancer: urogenital (3)	Genes common in: 1 cancer: liver (4)
Urogenital cancer	17	CDH5, POTEF, LILRB3	CES1, LAMB1	ALAD, ZNF605, CDH6, SERPING1, C1R		ATRN, CD163, IGDCC4 MASP1		ASCC, TPM2, FBLN1	
Urological cancer	8	CDH5, POTEF, LILRB3		ALAD, ZNF605, CDH6, SERPING1, C1R					
Breast cancer	7	CDH5, POTEF, LILRB3	CES1, LAMB1		PTPRG		S100A6		
Liver cancer	19	CDH5, POTEF, LILRB3	CES1, LAMB1	ALAD, ZNF605, CDH6, SERPING1, C1R	PTPRG	ATRN, CD163, IGDCC4 MASP1			GSR, SOD3 SAA4, SEMA7A

Comparison of the enriched genes representing our proteins in different types of cancer by analysis in the Ingenuity system biological database. For full name of the protein, see Table 3. ($n = 8$, $p < 0.05$).

stem cell-like neurons or neural stem cells are affected by sleep loss and maybe thereby memory consolidation, this might be worth exploring further. Thus, there is convincing evidence of functional correlations between adult-born neurons and memory consolidation and sleep (Koyanagi et al., 2019).

Complement C1r subcomponent (C1R) is a member of the peptidase S1 protein family and a proteolytic subunit in the complement system C1 complex (C1q together with C1r and C1s form the C1 complex) which is downregulated in our study. This complex is related to synaptic pruning by microglia (Stevens et al., 2007). REM sleep has shown to selectively prune and maintain new synapses under development and after learning (Li et al., 2017). Also C1q is assumed to be a C3-independent key mediator of age-related cognitive impairment (Hong et al., 2016) and where C1q, C3 and CR3 contributes towards an increased synapse loss in Alzheimer disease and memory consolidation (Ferreira et al., 2019).

ADP-ribosyl cyclase 2 or Bone marrow Stromal cell antigen 1 (BST1). This molecule facilitates pre-B-cell growth and thus, a reduction in this protein may compromise the immune system (Yamamoto-Katayama et al., 2002). BST1 is also linked to activate Ca^{2+}-release from intracellular stores or part of the regulation of calcium-mediated signaling, NAD+ nucleosidase activity, transferase activity, hydrolase, and cyclic ADP-ribose hydrolase activity (Yamamoto-Katayama et al., 2002). BST1, as we saw in human serum, was also found downregulated after 6 h SD in mouse brain entorhinal cortex (Thompson et al., 2010), however non-significant.

Coagulation factor VII (FA7) initiates the extrinsic pathway of blood coagulation and dysregulation of this coagulation factor protein can also lead to myocardial infarction (Shiraishi et al., 2017). A downregulation of this protein was found in our study after 6 h of sleep deprivation. Also, Thompson et al. (Thompson et al., 2010) showed a downregulation of Coagulation factor VII/FA7 in entorhinal cortex in the brain in mice after 6 h of sleep deprivation. Further, Pinotti et al. (Pinotti et al., 2010) showed a downregulation of this coagulation factor in the liver of mice after seven days of partial sleep deprivation. Thereby, a lower level of Coagulation factor VII/FA7 might increase the coagulation risk after sleep deprivation.

Laminin subunit beta 1 (LAMB1). This protein is involved in the organization of the laminar architecture of cerebral cortex (Radmanesh et al., 2013). It is probably required for the integrity of the basement membrane/glia limitans that serves as an anchor point for the end-feet of radial glial cells and as a physical barrier to migrating neurons (Radmanesh et al., 2013). We found a downregulation of Laminin subunit beta-1 (LAMB) protein after 6 h of sleep deprivation in human blood serum. Porter (Porter et al., 2012) showed a similar change on the transcription level in two other Laminin subunits (LAMB subtypes), Laminin beta 2 and Laminin gamma 1 after 24 and 72 h of sleep deprivation. This might indicate neuronal destabilization after sleep deprivation.

Extracellular superoxide dismutase [Cu-Zn] (SODE) protects the extracellular space from toxic effect of reactive oxygen intermediates by converting superoxide radicals into hydrogen peroxide and oxygen. We and

Table 8 The changed proteins in this study after 6 h of sleep deprivation (SD) compared to the same or similar genes or proteins found changed after SD or similar sleep loss conditions in tissue or body fluids reported by others. *

Proteins changed in our exp.			Genes and proteins found in the literature							
Symbol	**Name**	**Change**	**Symbol**	**Name**	**Species**	**Material**	**Method**	**Reference**	**Change**	**Condition**
ELTD1	EGF latrophilin and seven transmembrane domain-containing protein 1	UP	*ELTD1*	EGF, latrophilin seven transmembrane domain containing 1	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	UP	3,6,9 and 12 h of SD
H4	Histone H4	UP	H4 (protein)	Histone H4	R	Brain, cx		Cirelli et al. 2009	UP	sleep vs. short SD and sleep vs. waking
			HIST1H4A	Histone cluster 1, H4a	M	Brain, ent		Thompson et al. 2010	UP n.s	6 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	UP n.s	3,6,9 and 12 h of SD
			HIST1H4B	Histone cluster 1, H4b	M	Brain, ent		Thompson et al. 2010	DOWN n.s	6 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	UP n.s	3,6,9 and 12 h of SD
			HIST1H4C	Histone cluster 1, H4c	M	Brain, cx		Mackiewicz et al. 2007	UP n.s	3,6,9 and 12 h of SD
			HIST1H4D	Histone cluster 1, H4d	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
			HIST1H4H	Histone cluster 1, h4h	M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
			HIST1H4I	Histone cluster 1, h4i	M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	UP n.s.	3,6,9 and 12 h of SD
			HIST1H4J	Histone cluster 1, H4j	M	Brain, cx		Mackiewicz et al. 2007	UP n.s.	3,6,9 and 12 h of SD
			HIST1H4K	Histone cluster 1, h4k	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	UP n.s	3,6,9 and 12 h of SD
			HIST1H4M	Histone cluster 1, h4m	M	Brain, ent		Thompson et al. 2010	UP n.s	6 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	UP n.s	3,6,9 and 12 h of SD
			HIST4H4	Histone cluster 4, H4	M	Brain, ent		Thompson et al. 2010	UP n.s	6 h of SD
TPM2	Tropomyosin beta chain	UP	*TPM2*	Tropomyosin 2, beta	M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
			TPM1	Tropomyosin 1, alpha	H	Blood, whole		Möller-Levet et al. 2013	UP	1 wk. of 4 h SD
					R	Brain, hippo		Porter et al. 2012	UP	24 h and 72 h of SD
EST1	Liver carboxylesterase 1	UP	*CES1*	Carboxylesterase 1	M	Brain, ent		Thompson et al. 2010	UP	6 h of SD
LDH6A	L-lactate	UP	*LDHA*	L-Lactate dehydrogenase A	M	Brain,		Maret et al.	UP	6 h of SD

Table 8 The changed proteins in this study after 6 h of sleep deprivation (SD) compared to the same or similar genes or proteins found changed after SD or similar sleep loss conditions in tissue or body fluids reported by others. * *(Continued)*

Proteins changed in our exp.			Genes and proteins found in the literature							
Symbol	**Name**	**Change**	**Symbol**	**Name**	**Species**	**Material**	**Method**	**Reference**	**Change**	**Condition**
	dehydrogenase A-like 6A					whole		2007		
S10A6	Protein S100-A6	UP	S10A6 (protein)	Protein S100-A6	H	Urine		Becker et al. 2014	UP	OSA
			S100A4	S100 calcium-binding protein A4	M	Brain, ent		Thompson et al. 2010	DOWN	6 h of SD
			S100A1	S100 calcium-binding protein A1	R	Brain, hippo		Porter et al. 2012	DOWN	24 h and 72 h of SD
					M	Brain, ent		Thompson et al. 2010	DOWN	6 h of SD
			S100A16	S100 calcium-binding protein A16	M	Brain, cx		Mackiewicz et al. 2007	DOWN	3,6,9 and 12 h of SD
					M	Hyp		Mackiewicz et al. 2007	DOWN	3,6,9 and 12 h of SD
			S100A4	S100 calcium-binding protein A4	R	Brain, hippo		Porter et al. 2012	DOWN	24 h and 72 h of SD
					M	Brain, ent		Thompson et al. 2010	DOWN	6 h of SD
			S100B	S100 calcium-binding protein B	H	Blood, blood serum		Benedict et al. 2014	UP	8 h of SD
HEM2	Delta-aminolevulinic acid dehydRase	UP	*ALAD*	Aminolevulinate, delta-, dehydrase	R	Brain, hippo		Porter et al. 2012	DOWN	24 h and 72 h of SD
					M	Brain, ent		Thompson et al. 2010	DOWN	6 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	DOWN	3,6,9 and 12 h of SD
CADH5	Cadherin-5	UP	*CDH5*	Cadherin 5	M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
MYG	Myoglobin	UP	*MB*	Myoglobin	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
CADH6	Cadherin-6	UP	*CDH6*	Cadherin 6	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
IC1	Plasma protease C1 inhibitor	DOWN	SERPING1 (protein)	Plasma protease C1 inhibitor	H	Urine		Becker et al. 2014	DOWN	OSA
			SERPING 1	Serine (or cysteine) peptidase inhibitor, clade G, member 1	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
GSHR	Glutathione reductase, mitocondrial	DOWN	*GSR*	Glutathione reductase	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	DOWN n.s.	3,6,9 and 12 h of SD
PTPRG	Receceptor-type tyrosine-protein phosphatase gamma	DOWN	*PTPRG*	Protein tyrosine phosphatase, receptor type, G	R	Brain, hippo		Porter et al. 2012	DOWN	24 h and 72 h of SD
					M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
LIRB3	Leukocyte immunoglobulin-like receptor sub-family B member 3	DOWN	*LILRB3*	Leukocyte immunoglobulin-like receptor, subfamily B	M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
BST1	ADP-ribosyl cyclase 2	DOWN	*BST1*	Bone marrow stromal cell antigen 1	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD

Table 8 The changed proteins in this study after 6 h of sleep deprivation (SD) compared to the same or similar genes or proteins found changed after SD or similar sleep loss conditions in tissue or body fluids reported by others. * *(Continued)*

Proteins changed in our exp.			Genes and proteins found in the literature							
Symbol	**Name**	**Change**	**Symbol**	**Name**	**Species**	**Material**	**Method**	**Reference**	**Change**	**Condition**
FA7	Coagulation factor VII	DOWN	*F7*	Coagulation Factor VII	M	Liver		Pinotti et al. 2010	DOWN	7 days of partial SD
					M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
LAMB1	Laminin subunit beta-1	DOWN	*LAMB2*	Laminin, beta 2	R	Brain, hippo		Porter et al. 2012	DOWN	24 h and 72 h of SD
			LAMC1	Laminin, gamma 1	R	Brain, hippo		Porter et al. 2012	DOWN	24 h and 72 h of SD
SODE	Extracellular superoxide dismutase [Cu-Zn]	DOWN	SOD3 (protein)	Superoxide dismutase [Cu-Zn]	H	Urine		Becker et al. 2014	DOWN	OSA
			SOD3	Superoxide dismutase 3	R	Brain, hippo		Porter et al. 2012	UP	24 h and 72 h of SD
					M	Brain, cx		Thompson et al. 2010	UP n.s.	6 h of SD
			SOD1	Superoxide dismutase 1	H	Blood, whole		Möller-Levet et al. 2013	DOWN	1 wk. of SD (5.70 h, sleep per 24 h)
					R	Brain, hippo		Porter et al. 2012	DOWN	24 h and 72 h of SD
					M	Brain, cx		Mackiewicz et al. 2007	DOWN	3,6,9 and 12 h of SD
PCYOX	Prenylcysteine oxidase 1	DOWN	*PCYOX1*	Prenylcysteine oxidase 1	M	Brain, cx		Mackiewicz et al. 2007	DOWN	3,6,9 and 12 h of SD
					M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
SEM7A	Semaphorin-7A	DOWN	*SEMA7A*	Sema domain, immunoglobulin domain (Ig), and GPI membrane anchor, (semaphorin) 7A	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
			SEMA4C	Sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C	H	Blood, whole		Möller-Levet et al. 2013	DOWN	1 wk. of SD (5.70 h, sleep per 24 h)
			SEMA6B	Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6B	M	Brain, cx		Mackiewicz et al. 2007	DOWN	3,6,9 and 12 h of SD
SAA4	Blood serum amyloid A-4 protein	DOWN	*SAA4*	Blood serum amyloid A 4	M	Liver		Maret et al. 2007	UP	6 h of SD
					M	Brain, ent		Thompson et al. 2010	UP	6 h of SD
			Aβ	Amyloid- β	M	Brain, int. fluid		Kang et al.2009	UP	6 h of SD
C163A	Scavenger receptor cysteine-rich type 1 protein M130	DOWN	*CD163*	CD163 antigen	M	B		Thompson et al. 2010	UP n.s.	6 h of SD
FBLN1	Fibulin-1	DOWN	*FBLN1*	Fibulin-1	M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
			FBLN7	Fibulin-7	H	Blood, whole		Möller-Levet et al. 2013	DOWN	1 wk. of SD (5.70 h, sleep per 24 h)

Table 8 The changed proteins in this study after 6 h of sleep deprivation (SD) compared to the same or similar genes or proteins found changed after SD or similar sleep loss conditions in tissue or body fluids reported by others. * *(Continued)*

Proteins changed in our exp.			Genes and proteins found in the literature							
Symbol	Name	Change	Symbol	Name	Species	Material	Method	Reference	Change	Condition
MASP1	Mannan-binding lectin serine protease 1	DOWN	*MASP1*	Mannan-binding lectin serine peptidase 1	M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
			MASP2 (protein)	Mannan-binding lectin serine protease 2	H	Urine		Becker et al. 2014	DOWN	OSA
ASCC3	Activating signal cointegrator 1 complex subunit 3	DOWN	*ASCC3*	Activating signal cointegrator 1 complex subunit 3	M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
CRHBP	Corticotropin-releasing factor-binding protein	DOWN	*CRHBP*	Corticotropin releasing hormone binding protein	R	Brain, hippo		Porter et al. 2012	UP	24 h and 72 h of SD
					M	Brain, ent		Thompson et al. 2010	DOWN n.s.	6 h of SD
LOXL3	Lysyl oxidase homolog 3	DOWN	*LOXL3*	Lysyl oxidase-like 3	M	Brain, ent		Thompson et al. 2010	UP n.s.	6 h of SD
ATRN	Attractin	DOWN	ATRN (protein)	Attractin	H	Urine		Becker et al. 2014	DOWN	OSA
			ATRN	Attractin	R	Brain, hippo		Porter et al. 2012	UP	24 h and 72 h of SD
					M	Brain, ent		Thompson et al. 2010	DOWN	6 h of SD

* The 34 proteins, 6 are missing in this table: POTEI, IGHD, ZN605, POTEF, C1R and IGDC4. Because no same or similar genes or proteins have been found changed after SD or sleep/wake related conditions in the literature

another human study found this protein downregulated in blood serum and urine after 6 h of sleep deprivation and in children with obstructive sleep apnea, respectively (Becker et al., 2014). In several studies, reduced blood levels of antioxidants as SOD were reported in Alzheimer's disease, mild cognitive impairment and OSA (Baril et al., 2018; Zhou et al., 2016). Superoxide dismutase 1 (SOD1) has shown to be decreased in human whole blood after one week of 5.7 h sleep pr. 24 h and also a decrease in rat and mouse hippocampus and cortex after 24 and 72 h or 3, 6, 9 and 12 h of sleep deprivation respectively (Mackiewicz et al., 2007; Porter et al., 2012). Thus, sleep deprivation might reduce SODE's protective effect on extracellular space from toxic effect of reactive oxygen intermediates and thereby impair cognitive function.

Prenylcysteine oxidase 1-transcript (PCYOX) is involved in the degradation of prenylated proteins. PCYOX has a molecular function with an effect on ATPase-coupled chloride transmembrane transporter activity. It is essential for the proper cellular activity of numerous proteins, including Ras family GTPases and heterotrimeric G-proteins (Palsuledesai & Distefano, 2015). PCYOX was also found downregulated in mice after 3, 6, 9 and 12 h of sleep deprivation (Mackiewicz et al., 2007), in line with our findings on proteins in humans. If sleep deprivation via reduced PCYOX inhibits the suppression activity of Ras proteins, it might also be another factor contributing to a tumor promoting cellular environment.

Scavenger receptor cysteine-rich type 1 protein M130 (C163A) is an acute phase-regulated receptor involved in clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages and may thereby protect tissues from free hemoglobin-mediated oxidative damage (Subramanian et al., 2013). The soluble form (sCD163) may play an anti-inflammatory role and may be a valuable diagnostic marker for monitoring macrophage activation in inflammatory conditions (Catelas et al., 2018). We found a decrease in C163A after 6 h of sleep deprivation in human blood serum. Again, a decrease in C163A after sleep deprivation could be another factor contributing to increased oxidative damage.

Fibulin-1 (FBLN1) is incorporated into fibronectin-containing matrix fibers. It is believed to play a role in cell adhesion and migration along protein fibers within the extracellular matrix (Qing et al., 1997). It has also been implicated in a role in cellular transformation and tumor invasion, and appears to be a tumor suppressor (Hayashido et al., 1998). Thus, as the protein is downregulated in the present study it implies to have a role in tumor growth and migration. It may also play a role in haemostasis and thrombosis owing to its ability to bind fibrinogen and incorporate into clots (Tran et al., 1995).

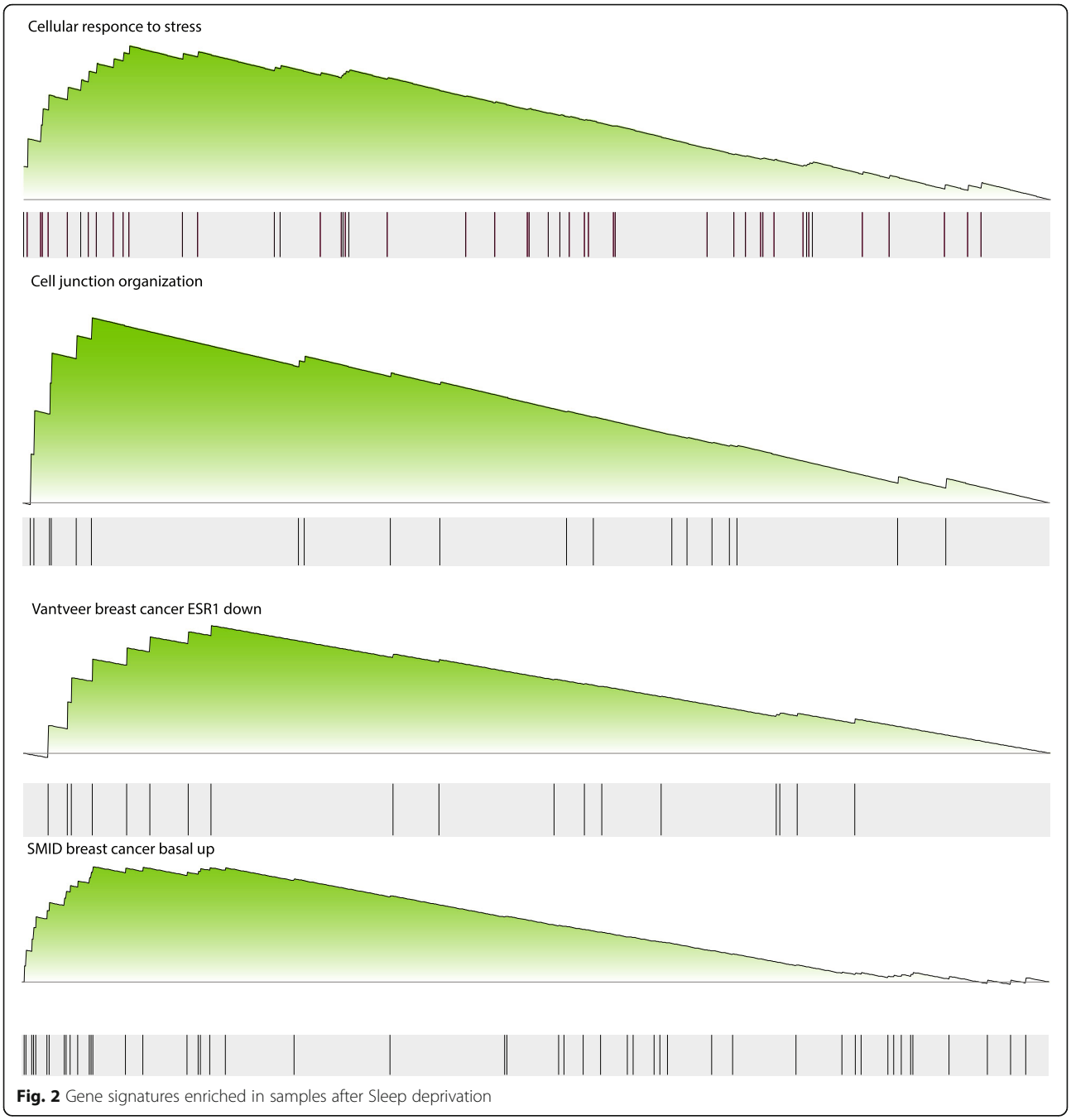

Fig. 2 Gene signatures enriched in samples after Sleep deprivation

Mannan-binding lectin serine protease 1 (MASP1) has a key role in innate immunity by recognizing pathogens through patterns of sugar moieties and neutralizing them (Endo et al., 2011). We found a decrease in MASP1 after 6 h sleep deprivation. Similar decrease is also shown in a human study in children with OSA (Becker et al., 2014). Also, an animal study by Thompson et al. (Thompson et al., 2010) showed a decrease of MASP1 in the entorhinal cortex in mice, although insignificant. These studies together could indicate that sleep deprivation can reduce the effectivity of the immune system.

Lysyl oxidase homolog 3 (LOXL3) acts as a regulator of inflammatory response by inhibiting differentiation of naive $CD4^+$ T-cells into T-helper Th17 or regulatory T-cells (Jeong et al., 2018). LOXL3 have also a role in developmental regulation, senescence, tumor suppression, cell growth control, and chemotaxis to each member of the family (Csiszar, 2001). We found a decreased level of LOXL3 after 6 h of sleep deprivation in human blood

Table 9 Proteins identified to be enriched in GSEA analysis

Gene Set Enrichment Analysis (GSEA) categories	Cell junction organization (6)	Cellular response to stress (12)	Smid Breast cancer basal up (14)	Vantveer breast cancer ESR1 down (8)
Gene name	CDH5	HIST1H4A	S100A6	DSC2
	KRT5	NEFH	NEFH	SOD2
	KRT14	RPS27A	KRT6A	WARS
	ACTB	PRDX1	KRT5	CDH3
	CDH6	DSC2	ACTA1	LDHB
	CDH3	SOD2	DSC2	PLTP
		KRT73	KRT14	KRT6B
		CA2	SPP1	KRT16
		THBS4	SOD2	
		PRDX2	WARS	
		CD93	S100A8	
		MPO	YWHAZ	
			CDH3	
			S100A9	

serum. Thereby, adding to the larger picture of reduced immune function as well as less tumor suppression and cell growth control.

Attractin (ATRN) is involved in the initial immune cell clustering during inflammatory response and may regulate chemotactic activity of chemokines. It has a critical role in normal myelination in the central nervous system (Duke-Cohan et al., 1998). We found a reduction in ATRN, which corresponds with the results of to two other studies after sleep deprivation (Thompson et al., 2010; Becker et al., 2014). Both studies were on the transcript level and protein level. Becker et al. (Becker et al., 2014) found the protein downregulated in OSA and Thompson (Thompson et al., 2010) found Attractin downregulated in mouse brain entorhinal cortex after 6 h of sleep deprivation at the transcript level. However, one study found Attractin upregulated after 24 and 72 h of sleep deprivation on transcript level in rat hippocampal tissue (Porter et al., 2012). A reduction in Attractin seems to influence immune, metabolism and nervous system functionality after 6 h of sleep deprivation.

Thus, the 16 significantly changes proteins in all our subjects can be categorized to the following functions: impaired coagulation, oxidative stress, immune suppression, neurodegenerative disorder, and cancer. Furthermore, according to our GO and GO-EA analysis several other proteins among the 34 significantly changed, also classified to the same categories.

Impaired coagulation

We observed changes in the blood serum level of seven proteins that are all important in blood coagulation: Epidermal Growth factor (EGF) latrophilin and seven transmembrane domain-containing protein 1 (ELTD1), liver carboxylesterase (EST1) and L-lactate dehydrogenase A-like 6A (LDH6A). These proteins were upregulated while Plasma protease C1 inhibitor (IC1), Receptor type tyrosine protein phosphatase gamma (PTPRG), Prenylcystein oxidase (PCYOX) and Attractin (ATRN) was downregulated. This has been found in prior sleep deprivation and/or sleep deprivation-associated studies to be changed accordingly (Cirelli et al., 2009; Mackiewicz et al., 2007; Maret et al., 2007; Thompson et al., 2010). Although these were mainly as gene expression changes either in human or animal-model studies in different tissue and/or body fluids (O'Callaghan et al., 2019).

Oxidative stress

Oxidative stress included cellular response to oxidative stress response and possibly cellular response to reactive oxygen species. We found changes in several of the same proteins related to oxidative stress in serum, as in several other studies after sleep fragmentation in OSA as well as in sleep deprivation and they changed in the same manner; S100A upregulated, Plasma protease C1 inhibitor (1C1), Extracellular superoxide dismutase [Cu-Zn] (SODE), Mannan-binding lectin serine protease 1 (MASP1) and Attractin (ATRN) downregulated (Mackiewicz et al., 2007; Moller-Levet et al., 2013; Porter et al., 2012; Thompson et al., 2010; Benedict et al., 2014; Becker et al., 2014).

Immune suppression

Complement activation was affected after 6 h sleep deprivation at night represented by the changed proteins; Plasma protease C1 inhibitor (IC1), Complement C1r subcomponent (C1R), Coagulation factor VII (FA7) and Mannan-binding lectin serine protease 1 MASP1 in Immune system and Complement Activation categories after classification in systems biological databases.

Also, humoral immune response including the proteins Plasma protease C1 inhibitor (IC1), Complement C1r subcomponent (C1R), ADP-ribosyl cyclase 2 (BST1) and Mannan-binding lectin serine protease 1 (MASP1) (downregulated) were seen in this study when

comparing the two systems biological databases Meta Core and Web Gestalt.

C163A Scavenger receptor cysteine-rich type 1 protein M130 (C163A) is an acute phase-regulated receptor involved in clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages (e.g. immune related) and may thereby protect tissues from free hemoglobin-mediated oxidative damage (Subramanian et al., 2013). The soluble form (sCD163) may play an anti-inflammatory role and may be a valuable diagnostic marker for monitoring macrophage activation in inflammatory conditions (Catelas et al., 2018). We found a decrease in C163A after 6 h of sleep deprivation in human blood serum and this could be another factor contributing to immunosuppression.

Attractin (ATRN) is involved in the initial immune cell clustering during inflammatory response and may regulate chemotactic activity of chemokines. A reduction in Attractin (ATRN) also seems to compromise immune, metabolism and nervous system functionality after 6 h of sleep deprivation (Thompson et al., 2010; Becker et al., 2014).

Neurodegenerative disease

Neuro-related proteins: Plasma protease C1 inhibitor (C1R) plays a role in synaptic pruning and miroglia function, Laminin subunit beta 1 (LAMB1) are associated with neuronal destabilization, Attractin (ATRN) are related to myelinization (Mackiewicz et al., 2007; Moller-Levet et al., 2013; Porter et al., 2012; Becker et al., 2014). All together reduced antioxidants as Extracellular superoxide dismutase [Cu-Zn] (SODE) or SOD has been found in both serum after sleep deprivation, and by others in urine from OSA-studies (Becker et al., 2014). Thus, this might possibly lead to cellular stress. It has also been related to neurodegenerative and neurodegenerative associated disorders (Alzheimer's disease, mild cognitive impairment and OSA (Owen et al., 2021; Koyanagi et al., 2019).

Cancer

After 6 h of sleep deprivation at night several of our 34 significantly changed proteins have also been associated with several common types of cancers as urogenital, urological, breast and liver cancers; Cadherin-5 (CDH5), POTE (Prostate, Ovary, Testes and Embryo) ankyrin domain family member F (POTEF), Laminin subunit beta-1 (LAMB1), Plasma protease C1 inhibitor (C1R), Attractin (ATRN), Protein S100-A6 (S100A6) and Extracellular superoxide dismutase [Cu-Zn] (SOD3). In addition, in our study Laminin subunit beta-1 (LAMB), Prenylcystein oxidase (PCYOX), merozoite surface protein 1 (MSP1), Fibulin-1 (FBLN1), Lysyl oxidase homolog 3 (LOXL3) are also cancer related.

Our changed proteins after sleep deprivation are also associated to breast cancer mRNA signatures and cell junction organization in Gene Set Enrichment Analysis (GSEA) as shown in Fig. 2 and Table 9. Finally, this corresponds to cancer incidences seen in longtime night shift workers and other shift workers (Haus & Smolensky, 2013; Kubo et al., 2006; Schernhammer et al., 2006).

In addition, molecular function categories using similar GO-EA analysis affected approximately half of the significantly changed serum proteins after 6 h SD at night. Protein Binding and Ion Binding categories represented by the serum protein changed in all 8 subjects including the following: C163A, FA7, IC1, LAMB1, LOXL3, MASP1, PTPRG, SODE, C1R, FA7, FBLN1, BST1, PCYOX, ATRN (downregulated) and CADH5, CADH6 and POTEF (upregulated). This point to cellular key functions being compromised after sleep deprivation.

Conclusions

In conclusion, our study suggests that sleep deprivation, at least in females, affects several known biological processes- and molecular function categories and associates to proteins that are also changed under pathological conditions like impaired coagulation, oxidative stress, immune suppression, neurodegenerative related disorder, and cancer.

Abbreviations

EA: Enrichment analysis; FDK: False discovery rate; iTraq: Isobaric tag for relative and absolute quantification; GO: Gene ontology; GSEA: Gen set enrichment analysis; HCD: Higher-energy collision density; LC: Liquid chromatography; MARS: Multi affinity removal system; MS: Mass spectre; NES: Normalized enrichment score; OSA: Obstructive sleep disorder; SCN: Suprachiasmatic nucleus

Supplementary Information

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Additional file 1: Table S1. Rules for the subjects one week before and during the experimental period.

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Authors' contributions

Conceived and designed the experiments: AAB. Performed the experiments: AAB. Analyzed of data: AAB, CD, FB, DSR, EB, LS, Contribution to the writing; AAB, LS, KB. All the authors have read and approved the final manuscript.

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Availability of data and materials

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the Pride partner repository with the dataset identifier PXD021004.

Declarations

Ethics approval and consent to participate

The study was approved by the Regional Ethics Committee (Regional Etisk Kommite (REK) Vest, numbers: 082.06 and 2019/253), informed consent was signed, and the participants were provided with sleep hygiene information (S1 and S2 appendix).

Consent for publication

Not applicable.

Competing interests

Noting to declare.

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