Whole blood gene expression signature in patients with obstructive sleep apnea and effect of continuous positive airway pressure treatment

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ABSTRACT
The molecular mechanisms of obstructive sleep apnea (OSA), in particular the gene expression patterns in whole blood of patients with OSA, can shed more light on the underlying pathophysiology of OSA and suggest potential biomarkers. In the current study, we have enrolled thirty patients with untreated moderate-severe OSA together with 20 BMI, age, and sex-matched controls and 15 normal-weight controls. RNA-sequencing of whole blood and home sleep apnea testing were performed in the untreated state and after three and twelve months of continuous positive airway pressure (CPAP) treatment. Analysis of the whole blood transcriptome of the patients with OSA revealed a unique pattern of differential expression with a significant number of downregulated immune-related genes including many heavy and light chain immunoglobulins and interferon-inducible genes. This was confirmed by the gene ontology analysis demonstrating enrichment with the biological processes associated with various immune functions. Expression of these genes was recovered after three months of CPAP treatment. After 12 months of CPAP treatment, the overall gene expression profile returns to the initial, untreated level. In addition, we have confirmed the importance of choosing BMI-matched controls as a reference group as opposed to normal-weight healthy individuals based on the significantly different gene expression signatures between these two groups.

1. Introduction
Obstructive sleep apnea (OSA) is a disease characterized by repeated total or partial upper airway collapse during sleep with maintained respiratory efforts. The resulting apneas and/or hypopneas lead to recurrent episodes of hypoxemia, sympathetic activation and micro arousals. OSA is considered a disease with a low grade of chronic inflammation and is associated with an array of co-morbidities such as hypertension and stroke (Gonzaga et al., 2015), metabolic disorders (Reutrakul and Mokhlesi, 2017), cognitive decline (Grigg-Damberger and Ralls, 2012), cancer (Martinez-Garcia et al., 2016), and increased overall mortality (Fu et al., 2017). Affecting 6–38 % of the adult population depending on its definition, OSA is a common disease and is found to be more common in men, the elderly, and obese individuals (Senaratna et al., 2017).

Notably, an alarming >80 % of individuals with OSA remain undiagnosed (Kapur et al., 2002; Young et al., 1997). The necessity of better diagnostics OSA and monitoring of treatment effects has therefore led to an intensive search for circulating molecular biomarkers. On a gene expression level, OSA appears to be a complex disease with multiple genes and pathways being affected and the common association of OSA and obesity further complicates the search for true OSA biomarkers (Arnardottir et al., 2009). During the last decade, there have been series of reports published based on analysis of either whole blood or peripheral blood mononuclear cells transcriptome using a microarray approach comparing OSA patients to controls (Chen et al., 2017; Hoffmann et al., 2007; Lin et al., 2014), the effect of continuous positive airway pressure (CPAP) treatment (Gharib et al., 2014; Peng et al., 2019;
Perry et al., 2013) or healthy volunteers exposed to the intermittent hypoxia (Polotsky et al., 2015). These studies have generated dissimilar results with up- or down-regulation of different genes involved in a variety of biological pathways, such as systemic and vascular inflammation, neoplastic processes, induction of apoptosis, and cell adhesion and communication.

The main aim of this study was to obtain the gene expression signature in whole blood of untreated patients with moderate and severe OSA and after three and 12 months of CPAP treatment.

2. Methods

2.1. Ethical approval

This study was conducted according to the standard of the Declaration of Helsinki and was approved by the Regional Ethics Committee on Human Research, Stockholm, Sweden (Dnr 2013/715-31/2 and Dnr 2014/768-32). Oral and written consent were obtained from all the study subjects. The study was registered prior to patient enrollment at clinicaltrial.gov #NCT 01976052 on the 21 October 2013 and it was conducted from October 2013 to April 2017.

2.2. Study subjects

This prospective longitudinal observational study consisted of three study groups: a) Adults, age 18–75 years and BMI < 40 kg/m² with newly diagnosed and untreated moderate or severe OSA accepting nightly CPAP treatment with no other medical condition apart from well-treated hypertension with no change in medication in the last 3 months, b) Age, sex and BMI matched non-OSA controls with an AHI < 5 and c) Healthy age and sex matched normal weight controls with BMI 19–25 kg/m² and an AHI < 5. Exclusion criteria were pregnancy, smoking/snuff, taking any antioxidant medication, or not being able to consent to the study.

Patients with OSA were recruited and diagnosed at a private outpatient clinic (Aleris Fysiologlab, Stockholm, Sweden). The matched and normal weight controls were recruited from the Stockholm population.

2.3. Study protocol

Home sleep apnea testing and blood tests were performed at three time points in patients with OSA – untreated at baseline and after three and 12 months of nightly CPAP treatment. In the age, sex and BMI matched controls and normal weight controls home sleep apnea testing and blood tests were performed at 0 and 12 months.

2.4. Sleep apnea diagnosis and CPAP treatment

Patients and controls performed home sleep apnea testing (Emblerta®, Embla, Reykjavik, Iceland or NOX T3TM, Nox Medical, Reykjavik, Iceland) including continuous recordings of thoracic and abdominal movements, nasal airflow via a nasal cannula connected to a pressure transducer, peripheral oxygen saturation, heart rate and body position (Christensson et al., 2019, 2018). The scoring of the sleep test was performed manually by a certified sleep staff according to the international guidelines (Berry et al., 2012). An obstructive apnea was defined as ≥90% reduction of airflow compared to baseline for ≥10 s with maintained thoracic and abdominal movements and a hypopnea as a reduction of ≥30% of airflow compared to baseline for ≥10 s together with maintained thoracic and abdominal movement and a desaturation of ≥3% (Berry et al., 2012). Total sleep time was estimated from the recordings. The average number of apneas or hypopneas per hour of sleep determined the apnea-hypopnea index (AHI) and the average number of ≥3% oxygen desaturations per hour of sleep determined the oxygen desaturation index (ODI) (Berry et al., 2012). An AHI < 5 was classified as non-OSA, AHI 5–14.9 as mild OSA, AHI 15–30 as moderate OSA and AHI > 30 as severe OSA. Data regarding the use of the home CPAP device (S9 AutoSetTM, ResMed, Sydney, Australia) during the study was collected from the CPAP software (ResScanTM, ResMed, Sydney, Australia).

2.5. Blood sample collection and isolation of ribonucleic acid (RNA)

After overnight fasting, blood samples were taken at 07:00–09:00 am. Two PAXgene™ blood RNA tubes (Qiagen, Hombrechtikon, Switzerland) were filled with 2.5 mL of blood. The tubes were then gently tilted 12 times and thereafter left in an upright position at room temperature for 72 h, then stored at –20 °C for 48 h and finally at –80 °C until processing. Extraction of total RNA was performed according to the manufacturer’s instructions. The samples were then treated with DNase, followed by RNA clean-up according to the manufacturer’s protocol by using RNA Clean-Up and concentration kit (Norgen Biotek Corp., Thorold, Canada). RNA quality control (Bioanalyzer, Agilent) reported RNA Integrity Number (RIN) values between 6.2 and 7.5.

2.6. RNA sequencing

The libraries for RNA sequencing were prepared using the Illumina TruSeq® Stranded Total RNA Library Prep Globin (Illumina Inc., San Diego, California, USA) that removes ribosomal RNA and globin mRNA and sequenced using the NovaSeq6000 system (Illumina Inc., San Diego, California, USA) at the Science for Life Laboratory, Stockholm, Sweden with a read length 2 × 50 bp giving >40 M reads per sample.

The raw sequence data were subjected to quality check using MultiQc v1.6. Reads were aligned to the GRCh37 human reference genome using short read aligner STAR v 2.7.3a. CPM (counts per million) values were calculated using Cufflinks 2.1.1. Data normalization and differential gene expression analysis were performed with Bioconductor R software package edgeR (Robinson et al., 2010) using Degust (http://vi ctorian-bioinformatics-consortium.github.io/degust/). The value of 0.5 CPM was chosen as a cut-off margin for filtering out the low-expression genes. The fold change threshold was ≥±1.5, false discovery rate, ≤0.05) for all the comparison groups.

Gene set enrichment analysis was conducted using two different online tools: Enrichr (https://amp.pharm.mssm.edu/Enrichr/) (Kuleshov et al., 2016) and David (david.ncifcrf.gov), v. 6.8 (Jiao et al., 2012).

Heatmaps visualizing hierarchical cluster analyses of gene expression and principal component analyses (PCA) plots were produced using Qlucore Omics Explorer software 3.5 (Qlucore, Lund, Sweden).

2.7. Quantitative PCR (qPCR)

Complementary DNA was synthesized utilizing the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Eugene, OR, USA) and oligo (dT) primers. The resulting cDNA amplified using Applied Biosystems 7500 Real-Time PCR System and the following TaqMan probes: interleukin 3 receptor (IL3RA), RNA component of mitochondrial RNA processing endoribonuclease (RMRP), interferon alpha-inducible protein 27 (IFIT2), interferon alpha-inducible protein 6 (IFI6), interferon-induced protein with tetraticopeptide repeats 1 (IFI1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Fisher Scientific, Gothenburg, Sweden). All samples were amplified in triplicates. The relative abundance of each transcript was estimated according to the 2^ΔΔCt method using GAPDH as a housekeeping reference gene.

2.8. Statistics

Normally distributed continuous variables (age, BMI, and lowest oxygen saturation) are presented as mean and standard deviation.
Continuous variables with skewed distribution (AHI and ODI) are presented as median and quartiles. Nominal values (sex) are presented as frequencies and percentages.

Statistical analysis of the quantitative PCR data was carried out using the one-way ANOVA option of the GraphPad Prism (version 8.0, San Diego, CA, USA) software. The threshold for statistical significance was set at \( p \leq 0.05 \) and the data are presented in the figure as means ± SD.

The set of statistical methods used for the analysis of RNA-seq data is described in the RNA sequencing section.

3. Results

3.1. Study subject characteristics

Enrolled were 30 patients with untreated moderate or severe OSA, 20 BMI, age and sex matched controls and 15 normal weight controls. The selection of the patients with OSA and the age, sex, and BMI matched controls is summarized in the flowchart (Fig. 1). Due to either cessation of CPAP therapy in some patients or failed sequencing of certain samples, the final analysis included 10 OSA patients with available RNA sequencing data for all three time points and 10 age-, sex- and BMI-matched non-OSA controls. There was a good match between the OSA patients and the matched controls in terms of age, sex and BMI (Table 1). In addition, six patients with documented excellent CPAP usage (>95% of possible nights between the third and twelfth month) were compared with six matched controls (data are presented in the supplemental section: Supplementary Tables S1–3, Supplementary Figs. S1–3). Finally, 19 matched controls were compared with 11 normal weight controls.

3.2. Differential gene expression in non-treated and CPAP-treated patients with OSA

Fig. 2 presents data on the number and temporal kinetics of differentially expressed genes (top 25 downregulated genes are presented in Table 2 and the complete list of differentially expressed genes can be found in Table S4) demonstrating the increased numbers of downregulated genes and decrease of upregulated genes after three months of nightly CPAP treatment and return to the 0 month level after 12 months treatment.

For more in-depth scrutiny of these longitudinal data, we have analyzed clustering of the samples at each of the studied time points using principal component analysis (PCA). The PCA plot shows that despite certain heterogeneity, samples are clustered into two distinct groups. One cluster consists of samples from patients with OSA after three months of CPAP treatment and age, sex and BMI matched controls and the other cluster contains a mix of samples from both untreated patients with OSA and after 12 months of CPAP treatment indicating similar gene expression profiles at these time points (Fig. 3A). This trend was supported by the analysis of hierarchical clustering of samples and genes based on their expression fold change values (Fig. 3B). Again, in the heat map, despite certain heterogeneity, the samples from 0 and 12 months tend to cluster together and are separated from the 3 months’ group (Fig. 3B).

In depth analysis of the heat map (Fig. 3B) and the source list of differentially expressed genes (Table 2, Table S4) reveals an interesting pattern. A considerable fraction of downregulated genes in untreated patients, consists of genes with an explicit immune function including various immunoglobulins, more specifically genes encoding for variable domains of different heavy and light chains (Table 2, Table S4) and these form a distinct cluster on the heat map (indicated by an arrow in Fig. 3B). Other immune-related top downregulated genes include IL3RA (interleukin 3 receptor), a group of interferon-inducible genes (IFI27, IFI6, IFIT1, MX1, OAS1, RSAD2), and also HLA-DQA1 representing α subunits of MHC class II cell surface receptors found on antigen-presenting cells and also shown to be activated by interferon (Boss, 1997; Giroux et al., 2003). Interestingly, as in the previous analyses, the expression of these genes largely returns to the same level as the matched controls after three months of CPAP treatment and, again reduced to nearly the same levels after 12 months (Table 2, Table S4).

This trend is illustrated by the temporal changes in the expression of immunoglobulin genes as well as interferon gamma-inducible genes as presented in Fig. 4A and B.
Top 25 most down-regulated genes at 0, 3 and 12 months of CPAP treatment compared to matched controls. The complete list of differentially expressed genes is presented in Table S3.

Untreated OSA patients

| Gene name | Description | FC | Gene name | Description | FC |
|-----------|-------------|----|-----------|-------------|----|
| AHR | aryl-hydrocarbon receptor repressor | -4.14 | AHR | aryl-hydrocarbon receptor repressor | -5.75 |
| CD177 | CD177 molecule [Source:HGNC Symbol:Acc/HGNC:30072] | -3.97 | RASD2 | radical S-adenylyl methionine domain containing 2 | -3.47 |
| SIGLEC1 | sigleic acid binding Ig like lectin 1 | -2.83 | IFIT1 | interferon induced protein with tetratricopeptide repeats 1 | -2.64 |
| IGHG4 | immunoglobulin heavy constant gamma 4 (G4m marker) | -2.91 | ZNF624 | zinc finger protein 624 | -2.60 |
| SIGLENC | sigleic acid binding Ig like lectin 1 | -2.83 | FCGR1A | Fc fragment of IgG receptor Ia | -2.61 |
| IFIT1 | interferon induced protein with tetratricopeptide repeats 1 | -2.76 | FGHI domain only 2 | -2.55 |
| IGHG3 | immunoglobulin heavy constant gamma 3 (G3m marker) | -2.75 | SIGLEC1 | sigleic acid binding Ig like lectin 1 | -2.49 |
| IGLV6 | immunoglobulin kappa variable 6–21 (non-functional) | -2.75 | SIGLENC | sigleic acid binding Ig like lectin 1 | -2.43 |
| IGLV6 | immunoglobulin kappa variable 6–21 (non-functional) | -2.75 | SIGLEC1 | sigleic acid binding Ig like lectin 1 | -2.43 |
| IGLV6 | immunoglobulin kappa variable 6–21 (non-functional) | -2.75 | SIGLENC | sigleic acid binding Ig like lectin 1 | -2.43 |

OAS; obstructive sleep apnea. CPAP; continuous positive airway pressure. FDR <0.05 for all genes presented in the table.

Table 3

The pathway analyses found only a handful of statistically significant and not related to each other GO terms (results not shown).

Analysis of the RNA-sequencing data from the selected six patients with excellent compliance to CPAP treatment generated a similar temporal pattern of changes in gene expression and similar sets of down-regulated immune-related differentially expressed genes and GO terms as described above for the larger group of OSA patients (Tables S1–3, Figs. S1–2). The PCA plot and heat map demonstrate an even better separation of samples from different time points, which is probably the reflection of a more homogenous group of patients with excellent
Fig. 3. Analysis of the differential gene expression in the whole blood of OSA patients.  
A. Principal component analyses (PCA) of the gene expression data (gene Counts-Per-Million, CPM) in 10 patients with OSA. The 0 and 12 months samples are clustered together whereas matched control samples and samples from the 3 months’ time point form a separate cluster. Both clusters are enclosed by the dotted line circles. B. Heat maps of differentially expressed genes based on the fold change (FC) values (FC ≥±1.5, FDR ≤0.05) showing hierarchical clustering of genes and samples in 10 patients with OSA. The color code bar indicates the FC range (red, upregulated, and blue, downregulated genes). Arrows indicate a group of immunoglobulin and interferon-inducible genes (see Fig. 4).

Fig. 4. Temporal kinetics of the differential expression of the heavy and light chain immunoglobulins (A) and interferon-inducible (B) genes. Each gene is indicated by a unique color line and a corresponding gene symbol. Gene descriptions for each of these immunoglobulins and interferon-inducible genes can be found in Table S4.

Table 3  
Top 10 most enriched Gene Ontology (GO) terms (biological processes) for downregulated genes at 0 and 12 months of CPAP treatment compared to matched controls. The complete list of enriched GO terms is presented in Table S5.

| Untreated OSA patients | After 3 months of CPAP treatment | After 12 months of CPAP treatment |
|------------------------|----------------------------------|----------------------------------|
| Biological processes   | Adjusted p-value                | Biological processes            | Adjusted p-value                | Biological processes            | Adjusted p-value                |
| cellular response to type I interferon | 1.11E-09                        | cellular response to type I interferon | 1.14E-04                        | complement activation, classical pathway | 2.83E-10                        |
| type I interferon signaling pathway | 1.11E-09                        | type I interferon signaling pathway | 1.14E-04                        | humoral immune response mediated by circulating immunoglobulin | 2.83E-10                        |
| complement activation, classical pathway | 1.11E-09                        | negative regulation of viral genome replication | 3.95E-04                        | Fe-gamma receptor signaling pathway involved in phagocytosis | 2.83E-10                        |
| humoral immune response mediated by circulating immunoglobulin | 1.11E-09                        | response to type I interferon | 3.95E-04                        | Fe-gamma receptor signaling pathway | 2.83E-10                        |
| Fe-gamma receptor signaling pathway involved in phagocytosis | 1.18E-09                        | negative regulation of viral life cycle | 7.82E-04                        | Fe receptor mediated stimulatory signaling pathway | 2.83E-10                        |
| Fe-gamma receptor signaling pathway | 1.18E-09                        | regulation of viral genome replication | 7.82E-04                        | regulation of protein activation cascade | 2.77E-09                        |
| Fc receptor mediated stimulatory signaling pathway | 1.18E-09                        | regulation of complement activation | 2.77E-09                        |                           |                                |
| cytokine-mediated signaling pathway | 3.70E-09                        | regulation of humoral immune response | 2.96E-09                        |                           |                                |
| regulation of protein activation cascade | 9.08E-09                        | regulation of immune effector process | 2.96E-09                        |                           |                                |
| regulation of complement activation | 9.08E-09                        | regulation of acute inflammatory response | 4.06E-09                        |                           |                                |

OSA; obstructive sleep apnea. CPAP; continuous positive airway pressure.
compliance to the CPAP treatment (Fig. S3).

3.3. Validation of RNA-sequencing data

All of the above-mentioned analyses were based exclusively on RNA-sequencing data. We conducted a limited validation of these data using a qPCR approach on a number of selected, predominantly immune-related genes. Results presented in Fig. 5 demonstrate nearly identical (although in certain cases quantitatively different) trends in both RNA-sequencing and qPCR-produced data.

3.4. Effects of obesity on gene expression

Obesity is a strong risk factor for OSA and approximately 70% of the patients with OSA are obese (Romero-Corral et al., 2010). However, not all OSA studies take this factor into account, which might be the reason behind the many contradictory studies published.

To select the correct reference group for our patients with OSA we compared the whole blood gene expression in age, sex and BMI matched controls with normal-weight healthy volunteers. BMI of the two groups was significantly different, \( p < 0.0001 \) (mean BMI 27.2 \( \pm \) 2.6 kg/m\(^2\) and 23.8 \( \pm \) 0.4 kg/m\(^2\) respectively for the matched and normal weight controls) and there was no difference in sex or age (mean age 50.0 \( \pm \) 12.6 years and 49.0 \( \pm \) 10.8 years respectively).

Comparison of these two groups produced a list of 278 up- and downregulated genes (Table S6). Principal component analysis (PCA) and hierarchical clustering of differentially expressed genes (heat map) demonstrate an almost perfect separation of these two groups indicating explicit differences in the global gene expression (Fig. 6A and B). Gene ontology analysis detected a limited number of significantly enriched (adjusted \( p \)-value \( < 0.01 \)) GO terms mostly related to the general cellular regulation (downregulated genes) and energy metabolism (upregulated genes) related biological processes (Table S7).

4. Discussion

Here we present unique longitudinal data on whole blood gene expression in patients with OSA before and after nightly CPAP treatment with an extended gene expression analysis after a treatment period as long as 12 months. The main finding here is the explicit difference in the whole blood gene expression patterns between untreated patients with OSA and age, sex and BMI matched non-OSA controls. Furthermore, we also found that the gene expression was recovered to the level of controls after three months of nightly CPAP treatment. After 12 months of nightly CPAP treatment the gene expression returned to the pattern characteristic for the untreated state.

All of the analytical methods used including PCA, hierarchical clustering or pathway analysis indicate the same recurring pattern – the return of the gene expression profiles and pathways affected to the original, untreated state after one year of nightly CPAP treatment. This was found regardless of if the study subjects had a good (10 study subjects) or excellent (6 study subjects) documented compliance to their CPAP treatment between three and twelve months. In neither of the two groups was the CPAP compliance inferior at twelve months compared to three months. At the same time, both groups had a good reduction and a normalization of the AHI and an increase of the lowest saturation at both three and twelve months of nightly CPAP treatment. The reasons for the unexpected temporal fluctuations of gene expression patterns are not clear. One possible explanation is natural annual cycles in gene expression. Annual variations of gene expression and also blood cell composition have been previously shown in children and adults in both whole blood, peripheral blood mononuclear cells and adipose tissue (De Jong et al., 2014; Dopico et al., 2015; Goldinger et al., 2015). Although half of the OSA study subjects were recruited during the autumn (September to November), the recruitment of the other patients was evenly spread along the remaining three seasons. Therefore, the annual cycles in gene expression are not the likely factor contributing to the observed gene expression temporal patterns.

Whereas the initial analysis of RNA-sequencing data uncovered explicit quantitative temporal patterns of differential gene expression, a closer look at the lists of differentially expressed genes identified two groups of downregulated genes that are involved in several immune-related biological processes enriched in OSA patients (Tables 2 and S4). The first group consists of a number of mRNAs encoding for different immunoglobulin chains and the second group includes various interferon activated downstream genes (See Results, Fig. 4A and B, Tables 2 and S4). Interestingly, our unpublished data on circulating cytokine levels in the same set of patients with OSA versus age, sex and BMI matched controls show a nearly two-fold drop in interferon gamma levels in untreated patients with OSA, which might be the reason for the corresponding downregulation of interferon dependent genes as observed in the current study. A similar decrease of interferon gamma blood levels in OSA patients has been shown previously (Said et al., 2017). There is an intriguing possibility that such a decrease in circulating interferon gamma may also alter the expression of yet another group of genes, i.e. immunoglobulins. It has been known since long ago that interferons can act directly to modulate (enhance) immunoglobulin synthesis in human B lymphocytes (Härfast et al., 1981; Kim et al., 2011; Jong et al., 2014; Dopico et al., 2015; Goldinger et al., 2015).
indicates the FC range (red, upregulated, and blue, downregulated genes).

differentially expressed genes based on the fold change (FC) values (FC considered to be a low grad inflammatory disease, where for example c-reactive protein, interleukin 6 and TNF-ISOFORMS (Vazquez et al., 2015). in the synthesis of immunoglobulins as mediated by various interferon cytokine-driven activation of the adaptive immune response indicates a Kimoto et al., 2011). A more recent review that summarized the data on cytokine-driven activation of the adaptive immune response indicates a clear possibility of B lymphocytes’ activation with a corresponding boost in the synthesis of immunoglobulins as mediated by various interferon isoforms (Vazquez et al., 2015).

OSA causes an activation of the sympathetic nervous system and is considered to be a low grad inflammatory disease, where for example c-reactive protein, interleukin 6 and TNF-α repeatedly have been shown to be increased in patients with OSA compared to controls (Geovanini et al., 2018; Nadeem et al., 2013). Moreover, recent studies demonstrate that neutrophils, lymphocytes and hematocrit are independently associated with OSA (Fan et al., 2019) and there is an association of neutrophils and OSA severity (Geovanini et al., 2018; Rha et al., 2020). However, in most of these studies OSA patients have been compared with normal weighted controls, and after correction for BMI much of the increase in inflammatory markers were attributed to obesity.

Previous studies on the global gene expression in the whole blood as well as in isolated peripheral blood mononuclear cells in patients with OSA and also intermittent hypoxia produced an array of conflicting results (see Introduction). It is notoriously difficult to compare different clinical studies and the treatment methodologies, however it should be noted that neither of them has registered any significant modifications in the expression of genes related to the inflammatory/immune pathways (one exception: activation of toll-like receptor 2 as reported by (Polotsky et al., 2015)), although there are scattered reports on the activation of CRP, IL-6 (De Luca Canto et al., 2015; Kritikou et al., 2014; Yokoe et al., 2003). However, this study shows a strong inhibitory effect of OSA on the expression of large groups of genes immediately involved in the processes of adaptive immunity (immunoglobulins, HLA genes) and antiviral response (interferon inducible genes).

Our data demonstrating distinctly different gene expression patterns in the two control groups (normal weight and BMI-matched) (Fig. 6) emphasized the importance of choosing BMI-matched controls as a reference group for studying different aspects of OSA (including gene expression), which was often neglected before (Chen et al., 2017; Hoffmann et al., 2007; Lin et al., 2014; Perry et al., 2013). Gene set enrichment analysis demonstrates a pattern (Table S7) that is largely consistent with the previous studies on gene expression in whole blood of obese subjects versus normal weight controls showing upregulation in various major cellular pathways related to ribosomal protein synthesis, apoptosis, oxidative phosphorylation and oxidative stress (Ghosh et al., 2010; Homuth et al., 2015).

There are several strengths of this study. The OSA population is well defined, their CPAP usage was well documented and the controls were matched for age, sex and BMI. To our knowledge, the study has a novel design with a longitudinal approach not attempted before in such patient population, where the whole blood gene expression patterns were estimated in the same patients at three time points in the course of one year. However, there are also some confounding factors of the study. Our gene expression data from the whole blood represent a complex mix of mRNAs from the peripheral blood mononuclear cells and also a multitude of free and exosome encapsulated circulating RNA species. Currently it is difficult to find a consensus opinion concerning the choice of whole blood or peripheral blood mononuclear cells in gene expression studies. Although whole blood has a heterogeneous composition, it is easier to handle, whereas peripheral blood mononuclear cell isolation is more laborious, which may also introduce additional ex vivo modifications in the transcriptome (Joehanes et al., 2012). The majority of the gene expression studies in OSA patients were done using isolated peripheral blood mononuclear cells (Chen et al., 2017; Gharib et al., 2014; Lin et al., 2014; Polotsky et al., 2015) and two studies used the total blood RNA (Peng et al., 2019; Perry et al., 2013). However, assuming that the peripheral blood mononuclear cell and total blood gene expression data largely overlap, and taking into account that the peripheral blood mononuclear cells are the origin of most of the immune-related differentially expressed genes it can be suggested that the differential gene expression as observed in our study occurs predominantly in the blood immune cells.

5. Conclusion

The whole blood transcriptome is modified in patients with OSA. These changes are largely normalized after three months of CPAP treatment, but return to the untreated OSA gene expression pattern after 12 months of successful CPAP treatment. Functional analysis of the differentially expressed genes in untreated patients with OSA indicates a decline in immune-related functions as reflected by the lower immunoglobulins’ and other immune-specific genes’ expression.

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Declaration of Competing Interest

LIE has received lecture fees from Merck Inc Sweden (Stockholm, Sweden) and advisory fees from Abbvie Inc, USA. MJF has received lecture fees from Fisher & Paykel (Auckland, New Zealand). EC, SM, AE, ÅOM, and KAF have no conflicts of interest.

Authors’ contributions

EC - This author helped to design the study and performed the experiments, data and statistical analysis, interpreted the results of the experiments and wrote the manuscript.

SM - This author helped with the conception of the study and performed the data and statistical analysis, interpreted the results of the experiments and wrote the manuscript.

AE - This author with the conception of the study and helped to perform the experiments, data analysis, interpreted the results of the experiment.

ÅOM - This author helped with the conception of the study and to perform the experiments, interpreted the results of the experiments.

KF and LIE - This author helped to design the study, interpreted the results of the experiments, and wrote the manuscript.

MJF - This author helped to design the study and performed the experiments, data, and statistical analysis, interpreted the results of the experiments and wrote the manuscript.

All authors read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.respli.2021.103746.

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