Proteoforms of transthyretin - candidate biomarkers in diagnosis of obstructive sleep apnea

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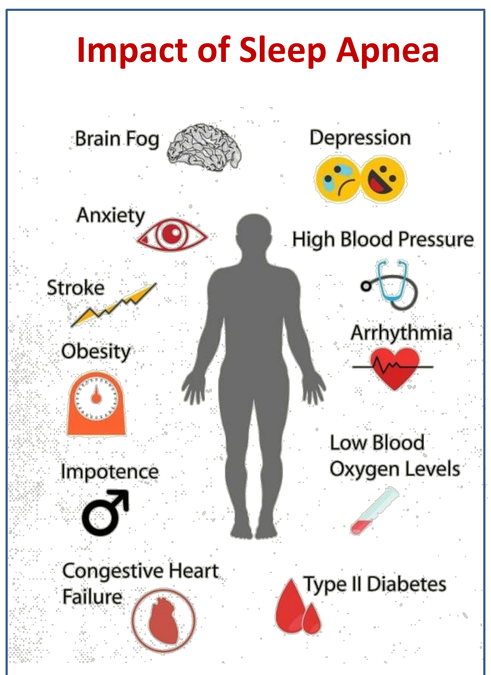
Background

Obstructive sleep apnea (OSA) is a common sleep-related breathing disorder which is characterized by recurrent occurrence of partial or complete closure of the upper airway during sleep, despite ongoing efforts to breathe. The majority of patients with OSA remain undiagnosed since most of them only come to the attention of a clinician when they complain of daytime sleepiness or when their bed partners report loud snoring or witnessed apnea episodes.

Epidemiological studies have indicated that **OSA affects 6–13% of the adult population**. OSA is multifactorial disease, also considered as metabolic syndrome, which **diagnosis in early stages is challenging** thus often remain **undiagnosed**. Recently was found connection between transthyretin (TTR) protein modifications present in human plasma samples and appearance of sleep apnea syndrome[1,2] . Mass Spectrometric Immunoassay (MSIA) was successfully applied previously on identification of and quantification of TTR variants present in human serum[3]. We took advantage on this powerful method to investigate possible modifications of TTR proteoforms in patients with OSAS.

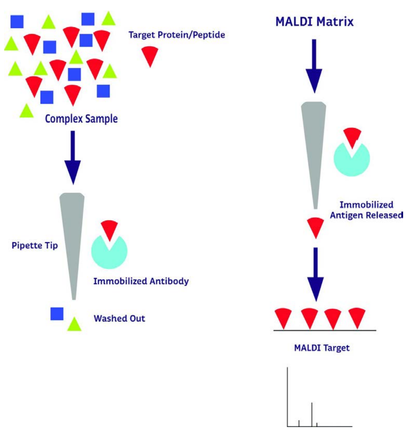


Methods

Biological specimen: Cohort of 84 male patients, with clinically suspected diagnosis of obstructive sleep apnea (OSA) syndrome were undergo polysomnography (PSG) – gold standard in diagnosis of OSA. Before PSG study, all subjects underwent a restricted diet during three days, including no drugs to induce sleep. The PSG was performed overnight at the sleep laboratory according to standard procedures. Blood from all examined individuals were collected before and after PSG, thus referred as evening and morning samples. Until analysis samples are stored at -80^{0}C. According to respiratory disturbance index (**RDI**) patients were classified as control (n = 20, diagnosed with primary snoring, RDI < 5/h) , mild (n = 30, RDI ≥ 5/h, but <15/h), moderate (n = 12, RDI ≥ 15/h, but <30/h) and severe OSA (n= 22, RDI ≥ 30/h).

Sample preparation: MSIA with TTR specific immunoaffinity columns were used to perform individual analysis of plasma samples of all examined subjects. All measurements were performed in two replicates. Prepared samples are analyzed on Bruker´s, Ultraflex MALDI-TOF-TOF mass spectrometry instrument.

Illustration of steps involved in MSIA sample preparation



Statistical data analysis: Statistical analysis was performed in R. Kruskal-Wallis (KW) test was used as a first approach to identify the parameters that should be further investigated. Parameters for which KW p-value <0.10 were subject to pairwise comparison among groups using Dunn's test. Linear regression was used to to estimate reçationship between parameters and RDI.

Results and Discussion

Identified transthyretin proteoforms:

A representative mass spectrum resulting from MSIA analysis of the plasma of the examined individuals is presented on Figure 1. Targeted protein is transthyretin (TTR). However, due to genetic mutations and post translational modifications TTR is appearing as multiple signal. The predominant form is cysteinylated TTRCys (MW=13,880.8 Da), followed by the native TTRn (MW=13,760.9 Da) and cysteinyl-glycine TTRCysGly (MW=13,943.9 Da)form. Also it can be noted in the mass spectra are signals assigned to oxidation form of TTRox (MW=13,792.8), sulfonation TTRs (MW 13,842.3 Da) and Cysteine-Glycine transformation TTRCys10Gly(MW=13,717.6 Da). All of these TTR variants occur as a result of the modifications of a single highly reactive cystein residue (Cys10). Its of the great importance to analyze this proteoforms due to the crucial role of the free Cys10 residue and possible involvement of pathophysiological factors that affect Cys 10 reactivity.

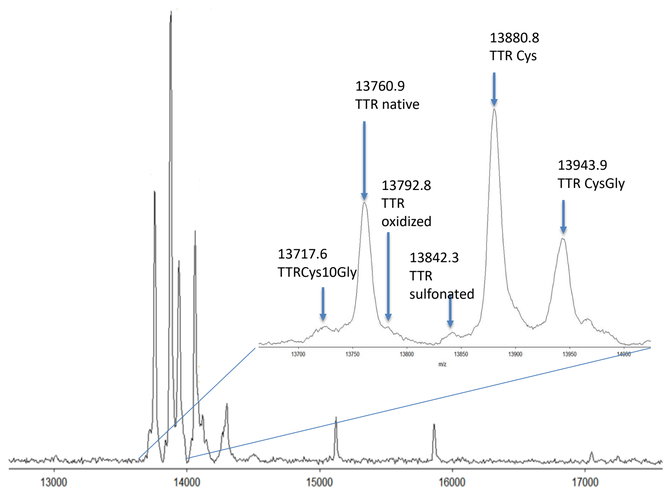


Fig. 1. Typical MS spectra of TTR proteoforms originating from human plasma samples

Data mining:

Intensities of the MS signals the TTR proteoforms were measured. Our goal was to investigate how these data modify related to OSA pathology. To achieve that we followed changes in proteoform expression before and after sleep (evening- morning variations). Also we observed changes of TTR variants longitudinally – between groups.

- Results of KW test of samples taken before PSG (evening) revealed that all proteoforms except TTRox are significantly downregulated in individuals with OSA when compared to snorer group (Fig. 2). However, Dunn test revealed that **TTRCys10Gly** and **TTRCysGly** distribute differently according to OSA severity with statistical significance (α=0.1).
- Levels of TTR variants in the morning samples are not differ between groups
- Correlation model exhibit statistically significant linear dependence of **TTRCys10Gly, TTRn** and **TTRCysGly** on RDI for significance level of 10% (Fig.3). In other words for unit increment of RDI, there is stat. significant decrease in value of protein signal intensity.
- Evening-morning differences are also stat. significant for the **TTRCys, TTRn, TTRs** and **TTRCysGly.** However, post-hoc Dunn test revealed that only evening-morning difference of **TTRCysGly** are statistically significant depending on OSA pathology level.
- Following differences on evening-morning TTR´s expression it noticed strong positive dependence on RDI for following TTR variants: TRCys, TTRn, TTRCysGly and TTRs. This findings suggest that in spite of breathing disturbance some reparation mechanisms are triggered toward maintenance of TTR-stasis.
- Most of the patients are obese, thus abdominal perimeter is one of the factors which is followed in routine physical examination of OSA suspected patients. We wish to test hypothesis that some of the TTR variants can be brought in correlation with abdominal parameter, offering the possibility to triage OSA suspected individuals before they are sent to PSG. On Fig.5 is presented correlation of the abdominal parameter on RDI. Cysteinil-Glycine variant of TTR in the samples taken before PSG, exhibited highest correlation with RDI and abdominal parameter hence suggested that can be consider as potential new biomarker for OSA.

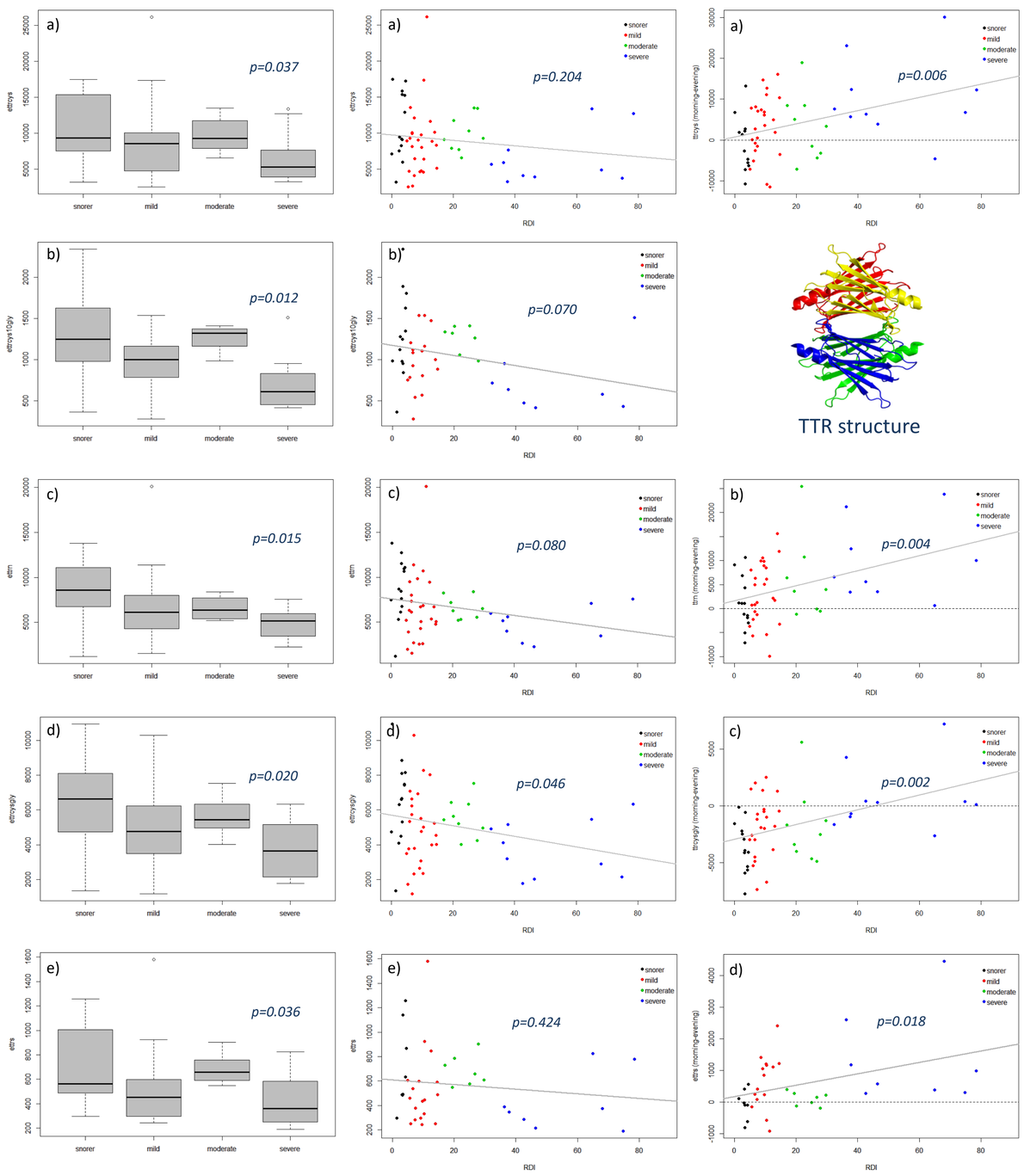


Fig. 2. Differences of TTR proteoforms expression in function of OSA severity in the samples collected in evening: a) TRCys, b)TTRCys10Gly, c)TTRn d)TTRCysGly and e)TTRs

Fig. 3. Linear dependence of TTR proteoforms on RDI in function of OSA severity in the samples collected in evening: a) TRCys, b)TTRCys10Gly ,c)TTRn d)TTRCysGly and e)TTRs

Fig. 4. Linear dependence of evening-morning difference of TTR proteoforms on RDI in function of OSA severity: a) TRCys, b)TTRn, c)TTRCysGly and d)TTRs

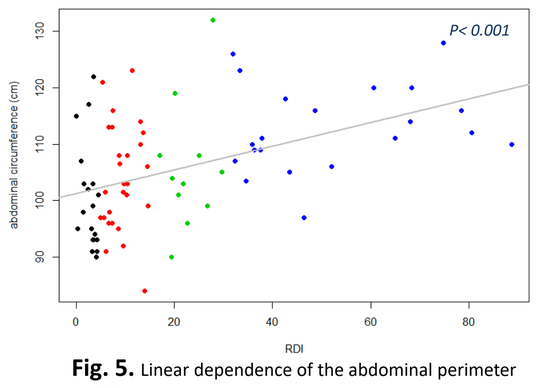


Fig. 5. Linear dependence of the abdominal perimeter on RDI

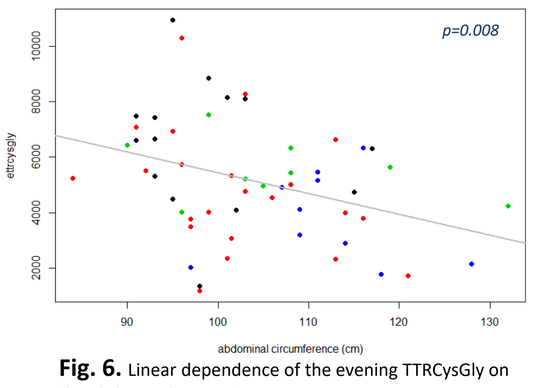


Fig. 6. Linear dependence of the evening TTRCysGly on the abdominal perimeter

Conclusion

Obstructive sleep apnea (OSA) is an underdiagnosed common public health concern causing deleterious effects on metabolic and cardiovascular health. Gold standard for diagnosis is polysomnography and it can be only done in hospitals and specialized sleep clinics. Recent studies found connection between TTR protein modifications present in human plasma samples and appearance of sleep apnea syndrome. In our work we investigated expression of TTR proteoforms in individuals with different severity of OSA. This findings revelad that before sleep 5 of totaly 6 identified TTR variants are significantly decreased comparing to control snorers, while cysteinyl-glycine modification (TTRCysGly) has strong significance in differentating OSA severity. Analysis of morning samples revealed that reparation mechanisms are triggered during sleep resulting in balanced levels of TTR proteoforms between all groups. Both, evening levels of individual TTR variants and evening-morning difference have significant dependence on RDI. However, change in TTRCysGly expression is highlighted because it significantly correlates with RDI values, OSA severity and abdominal perimeter. This findings make TTRCysGly new putative biomarker for screening of OSA patients and potentially great addition to further OSA diagnosis. To validate obtained results MSIA-TTR analysis on large cohorts are need. Pathophysiological mechanisms behind these changes on TTR is still to be elucidated.

References

1. *Bodez D, Guellich A, Kharoubi M, et al. Prevalence, Severity, and Prognostic Value of Sleep Apnea Syndromes in Cardiac Amyloidosis. Sleep. 2016;39(7):1333-1341.*
2. *Tan HL, Kheirandish-Gozal L, Gozal D. The promise of translational and personalised approaches for paediatric obstructive sleep apnoea: an 'Omics' perspective. Thorax. 2014;69:474–80.*
3. *Trenchevska O, Kamcheva E, Nedelkov D. Mass Spectrometric Immunoassay for Quantitative Determination of Transthyretin and its Variants. Proteomics. 2011;11(18):3633-3641.*
4. *Kishikawa M, Sass JO, Sakura N, Nakanishi T, et al. The peak height ratio of S-sulfonated transthyretin and other oxidized isoforms as a marker for molybdenum cofactor deficiency, measured by electrospray ionization mass spectrometry. Biochim Biophys Acta. 2002;1588:135–138.*

HUPO Dublin Ireland 17 – 20 September 2017
16th Human Proteome Organisation World Congress

Acknowledgment

To patients that voluntarily collaborated in this study. Project partially supported by Harvard Medical School-Portugal Program (HMSP-ICJ/0022/2011), ToxOmics - Centre for Toxicogenomics and Human Health (FCT-UID/BIM/00009/2013), FCT/Poly-Annual Funding Program and FEDER/Saúde XXI Program (Portugal) and postdoctoral fellowship SFRH/BPD/43365/2008 of Fundação para a Ciência e a Tecnologia(FCT),Portugal.