Metabolomic Mechanisms of Symptoms in Sleep Apnea and Cardiovascular Disease: Future Directions for Big Data

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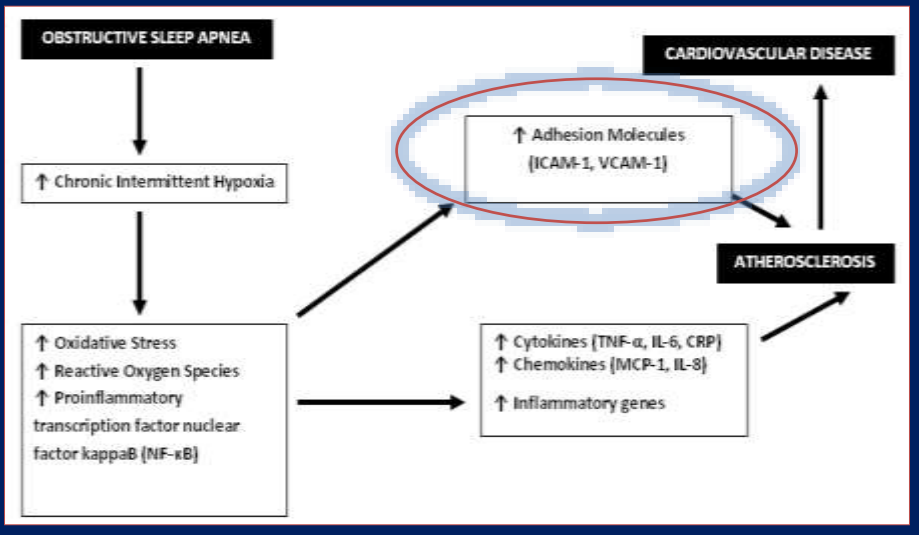
Outline

- Discuss research on understanding the underlying mechanisms (genetic/metabolomic) of sleepiness
- Next steps for big data in sleep research

Elevated Cellular Adhesion Molecules May Contribute to Cardiovascular Disease

Pak, VM, et al. Pack, AI. (2013). Sleep Med Rev. Feb;18(1):25-34.



Candidate gene analysis in the São Paulo Epidemiologic Sleep Study (EPISONO) shows an association of variant in *PDE4D* and sleepiness

Pak, V.M. et al. Pack, A.I., Tufik, S. (2018). Sleep Medicine. 47: 106-112.

Outline of study

- Background
  - Explored SNPs in candidate genes within oxidative stress, inflammatory, neuronal pathways and link to sleepiness.
- Methods
  - 918 subjects, ESS available, and genotyped
- Main Results
  - Novel association between the C allele of the rs12522161 SNP on PDE4D and a decreased likelihood of sleepiness.
- Conclusion
  - We present data for a novel genetic association with sleepiness for a SNP on the PDE4D gene, rs12522161.

Study procedures

- Representative sample of inhabitants were obtained via 3-stage cluster sampling technique in São Paulo, Brazil
  - Socioeconomic screening, random selection, and age divisions
- Full-night PSG performed on digital system (EMBLA S7000) at Sleep Institute, 80 bed unit.
- Blood samples collected morning after sleep study and DNA extracted.

São Paulo Epidemiologic Sleep Study (EPISONO)

- The sample consisted initially of 1,042 individuals who were part of EPISONO–from São Paulo, Brazil, conducted in 2007.

- The EPISONO examines sleep disturbances and their risk factors and contains sleep phenotyping and related data.

Main Results

- **Table 2**
  Nominally significant independent associations (after pruning*) with categorical sleepiness in adjusted analyses.

Gene	Chr.	SNP	MAF		PC and covariate[b] model	
			Cases ESS ≥ 10	Controls ESS < 10	OR (95% CI)[d]	p**
PDE4D	**5**	**rs12522161**	**18.8%**	**25.9%**	**0.64 (0.50, 0.81)**	**0.0002**
PDE4D	5	rs1445918	30.1%	23.2%	1.44 (1.15, 1.81)	0.0013
PDE4D	5	rs167161	40.9%	33.9%	1.36 (1.11, 1.66)	0.0029
PDE4D	5	rs35281	29.9%	36.3%	0.73 (0.59, 0.90)	0.0032
PDE4D	5	rs6874388	8.9%	6.0%	1.81 (1.19, 2.74)	0.0051

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PDE4D (chromosomal location 5q12)

- Gene belongs to super family of phosphodiesterases (PDE4 family)
- PDE4 is a family of enzymes in the hippocampus, frontal cortex, olfactory bulb and cerebellum
  - Responsible for degradation of 3',5'-cyclic AMP (cAMP)

Discussion and conclusion

- Strongest association with sleepiness was found on the C allele of the rs12522161 SNP on inflammatory gene PDE4D.
- Future studies are needed to replicate and determine functional effects of the SNPs discovered and clarify role of sleepiness.

Metabolomics, Sleepiness, and Sleep Duration in Sleep Apnea

- Daytime sleepiness, a common sequela of OSA is correlated with adverse cardiovascular outcomes
  - Understanding metabolomic mechanisms will provide insight
- Lack of studies examining metabolomic mechanisms

Diallo, I. & Pak, VM. 2020; Sleep and Breathing doi: 10.1007/s11325-019-01969-2

Lower plasma choline levels are associated with sleepiness symptoms

Pak VM, et al, Pack, A.I. (2018). Sleep Medicine. 44, 89-96.

Outline

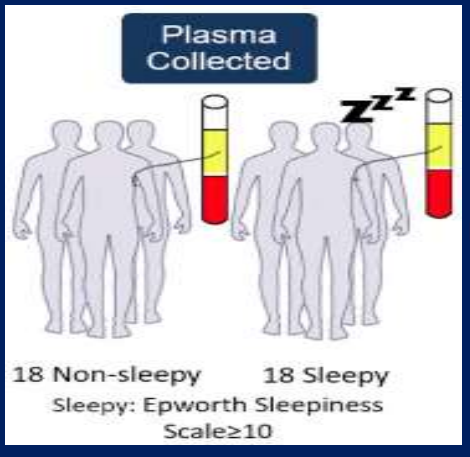
- Introduction
  - Daytime sleepiness predicts cardiovascular disease, although the mechanism is unidentified.
- Methods
  - Sleep study, blood samples of newly diagnosed treatment naïve OSA patients at U.Penn
- Main Results
  - Level of choline is significantly lower in sleepy subjects N = (18) compared with non-sleepy subjects (N = 18). Other metabolites were also identified.
- Conclusion
  - This pilot study is the first to show that lower levels of plasma choline metabolites are associated with sleepiness.

Participant Demographics

	Sleepiness			
	No (N = 18)	**Yes (N = 18)**	**Total (N = 36)**	**P-Value**
Age	41.44 (11.05)	43.33 (10.15)	42.39 (10.50)	0.60
Epworth Sleepiness Scale (ESS)	5.08 (2.20)	14.10 (3.53)	9.81 (5.43)	**<0.001**
Apnea Hypopnea Index, events/hr	8.2 (3–28.10)	10.05 (1–23.80)	8.2 (2.5–26.5)	0.64
BMI, kg/m^2	36.29 (9.88)	35.63 (9.09)	35.96 (9.36)	0.84
Total minutes sleep time	356.0 (300.0 – 422.2)	384.5 (357.0 – 434.1)	358.8 (325.0 – 428.2)	0.36
Gender				
Female	9 (50.00%)	9 (50.00%)	18 (50.00%)	0.99
Male	9 (50.00%)	9 (50.00%)	18 (50.00%)	
Smoking				
No	14 (87.50%)	11 (61.11%)	25 (73.53%)	0.13
Yes	2 (12.50%)	7 (38.89%)	9 (26.47%)	

Methods

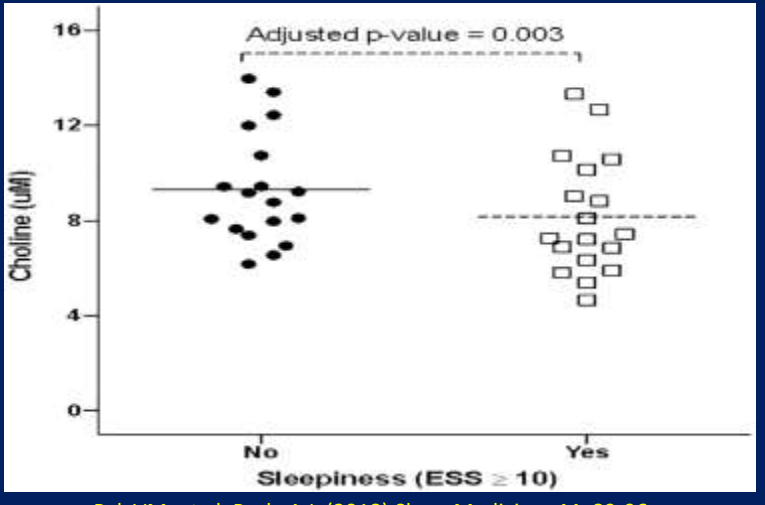
- An exploratory case–control sample of 36 subjects at the U.Penn Sleep Center.



Panels analyzed

1. Acylcarnitines
2. Ceramides (Cer)
3. Trimethylamine N-oxide (TMAO)
4. Neurotransmitters and Amino Acids

Differences in choline in subjects with sleepiness versus those without



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Metabolites linked to sleepiness (P<0.1)

- **Choline** (P<0.003)
- Alpha-Aminoadipic-acid
- Sphingosine 1 Phosphate
- Isovalerylcarnitine
- Aspartic Acid
- C16-Ceramides
- C14-Ceramides
- C18:1-Ceramides
- Sphinganine
- Propionylcarnitine

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Limitations and strengths

- Limitations
  - Small sample (36 subjects)
  - Measurement of sleepiness phenotype is subjective
- Strengths
  - First study exploring metabolic profiles of subjects among those with and without sleepiness
  - Robust clinical information available

Conclusions

- Further exploration of relevant metabolites will guide targeted symptom management.

Big Data & Sleep Research

- Early collaboration across institutions and health systems and data harmonization is needed
  - Discuss biomarker collection and storage procedures
  - Measurement consistency
  - Time to assay
  - Lab analysis procedures

Big Data & Sleep Research

- Building of infrastructure will require close collaboration with sponsors, health systems, and institutions
  - discuss logistics of data consistency and accessibility

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