Stress induced miRNA changes in depression: peripheral biomarker or pathophysiology?

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Background

• **Stress** occurs on social, psychological and metabolic levels. If the neuro-immuno-endocrine system can not react appropriately to the changing circumstances, maladaptive diseases occur such as major depression (MD).

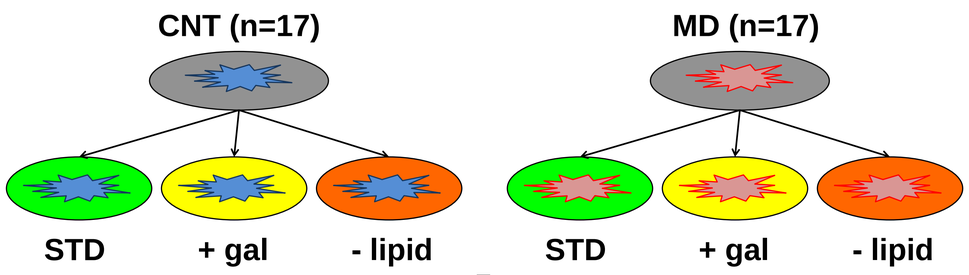
• The lifetime prevalence of **MD** is 20%. It is a multifactorial disease: the individual vulnerability depends on environmental (stressful life events) and genetic factors (gene mutations and suspected complex gene expression regulatory dysfunction)[1].

• **MicroRNAs** are small, non-coding RNAs, which play role in the regulation of posttranscriptional gene expression by inducing destabilisation, degradation or storage of the target mRNAs.

• MicroRNAs have special role in neural plasticity (learning), maintenance of the circadian rhythm and mechanism of antidepressants[2].

• **Fibroblasts** are considered as *in vitro* neuron models since their receptor profile and signal transduction is very similar to the brain tissue[3].

Subjects and Methods



3. total and small RNA isolation (miRVana Kit, Ambion)

4. RNA pooling (n=4-5 / group)

5. DNA microarray (HT HG-U133+ PM Array, Affymetrix)

6. miRNA expression profiling (miRNome SYBR-green PCR Kit, Qiagen)

7. qPCR validation of certain miRNAs in individual samples

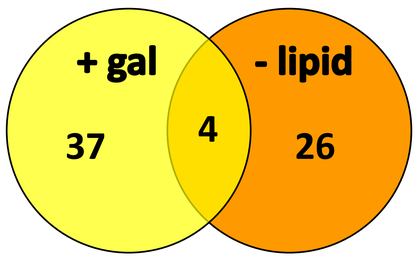
1. Fibroblast cultures from skin biopsies of
- patients with major depression (MD)
- controls (CNT)

2. Metabolic stress treatment[4] (1 week)
- STD = standard medium
- + glu = glucose deprived, galactose enriched
- - lipid = lipid reduced, cholesterine deficient

CNT	age (yrs, avg)	MD	age (yrs, avg)
CNT1	30-48 (37,5)	MD1	29-46 (36,5)
CNT2	27-40 (32,2)	MD2	26-31 (31)
CNT3	44-52 (49)	MD3	43-52 (49,7)
CNT4	20-25 (21,8)	MD4	22-23 (22,4)

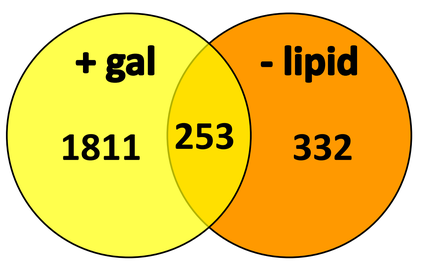
8. Data mining – statistical analysis
- $|ALR| \geq 0.378$; $|ddCt| \geq 0.583$; $p<0.05$
- analyses of correlation between the mRNA and miRNA alterations

Our Questions and Results

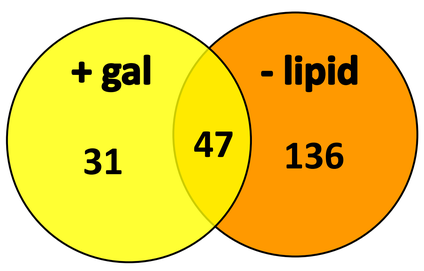


microRNA stress response in CNT

1. Which microRNAs and genes are involved in the healthy stress reaction?
Only 4 microRNAs are affected by both metabolic stress treatments. One of these is miR-146b-5p which regulates the NFκB pathway through targeting IRAK-1 mRNA[5]. Pearson correlation showed that the overall gene expression profile after the two different treatment are similar to each other (R^2=0.51 and 0.43).

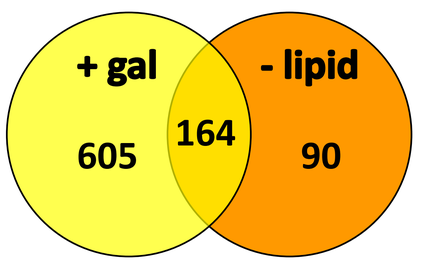


mRNA stress response in CNT

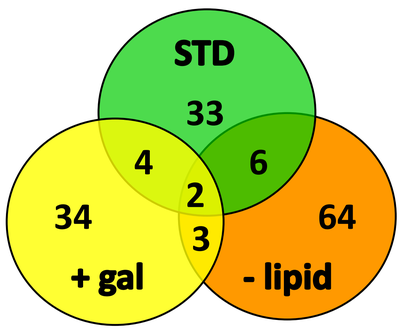


microRNA stress response in MD

2. Which microRNAs are affected by stress in MD samples?
The PCR microRNome results show that 183 microRNAs changed in the lipid reduced environment. 9 of these are known to play role in stress reaction and neural plasticity (miR-16, 18, 21, 30e, 34a, 124, 132, 134, 376).

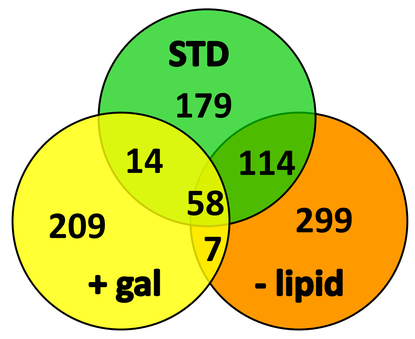


mRNA stress response in MD



microRNA profile in MD compared to CNT

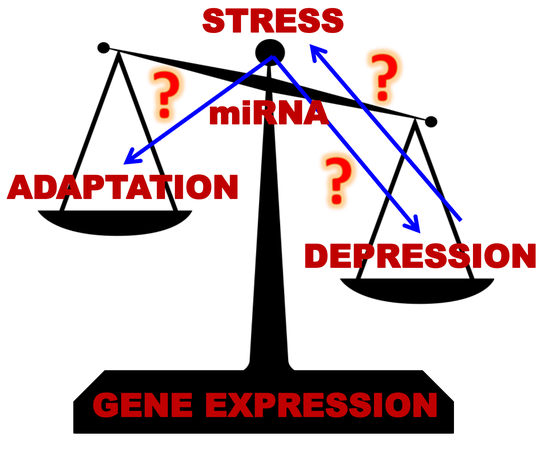
3. How the microRNA and gene expression profile in MD differs from CNT?
45 microRNAs are differently expressed in the MD samples under STD circumstances.
Previously 3 of these were referred as depression-associated microRNAs (miR-18, 34a and 132)[6].



mRNA profile in MD compared to CNT

stress induced pathways in CNT
classical complement pathway
AKT signaling pathway
tumor suppressor ARF inhibits ribosomal biogenesis
complement pathway

4. Which pathways are induced by metabolic stress?
Both stress treatments activated mostly immun regulation and cell cycle related gene-pathways both in CNT and MD groups. These changes highlight the possible neuro-immuno-endocrine pathomechanism in MD.



stress induced pathways in MD
antigen dependent B cell activation
hiv induced T cell activation
activation of csk by PKA inhibits signaling through T cell receptor
LCK and FYN tyrosine kinases in initiation of TCR activation
cytokines and inflammatory response

Discussion

1. We examined the microRNA profile of MD patients' fibroblasts first in the literature → new possible diagnostic and therapeutic **biomarker**s.
2. The microRNA stress response pattern of CNT is similar after the two different metabolic stresses → proof of an evolutionary fixed **adaptation scheme**.
3. Individual variability of the stress reaction among CNTs → originates from **genetic diversity** of CNT population.
4. MD group reacts on stress differently on the level of gene expression (mRNAs) and gene regulation (microRNAs) → MD is a **maladaptive pathway** where the genetically impaired stress response sensitises the individuum for the environmental stressors.
5. We found similar microRNA stress reaction on populational level in MD → underlies the role of **hereditary factors** which may occur not only on the level of single gene polymorphism but also in complex gene expression regulation.
6. Fibroblasts can be easily gained and cultured and have similar intracellular pathways as neurons → new potential human, *in vitro* depression-stress **model**s.

References

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MAGYARORSZÁG MEGÚJUL
A projekt az Európai Unió támogatásával, az Európai Szociális Alap társfinanszírozásával valósul meg.
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