The pathways of Rett syndrome revealed by different methods for pathway and network analysis

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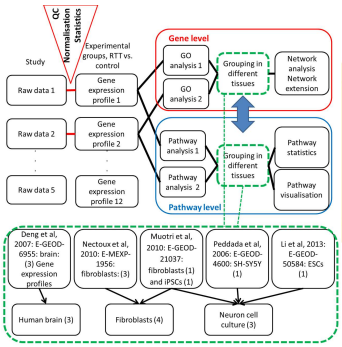
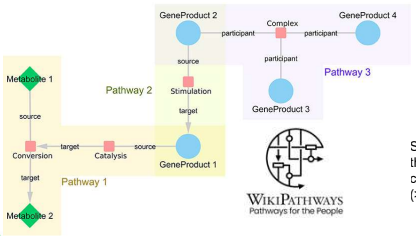
Background: Rett syndrome is a rare genetic disorder caused by a loss of function mutation in MECP2, an important regulator of gene expression especially, but not only, in neurons. Due to the multi-functionality of MECP2 there are many downstream pathways which are interesting for understanding the pathophysiology of Rett syndrome, and allowing a search for drug targets. Using omics data and analysing it in terms of biological pathways and networks allows a holistic view of the influence of MECP2. In the past years several methods have been developed, which we will demonstrate here.

Methods: Study 1 Transcriptomics microarray data was collected previously published studies of which differentially expressed gene lists were extracted (Ehrhart et al. 2019). **For Study 2** (Miller et al. 2019) the original dataset is from Lin et al. (2016). Overrepresentation analysis of biological pathways was generally done in PathVisio software using the pathway database WikiPathways (including Reactome pathways). For active module analysis in study 2, first, the whole WikiPathways database was used to construct one large network and, second, active modules based on differently expressed genes were identified using the Cytoscape app jActiveModules.



Study 1 design: the raw transcriptomics data of 5 independent studies was collected and reanalyzed.



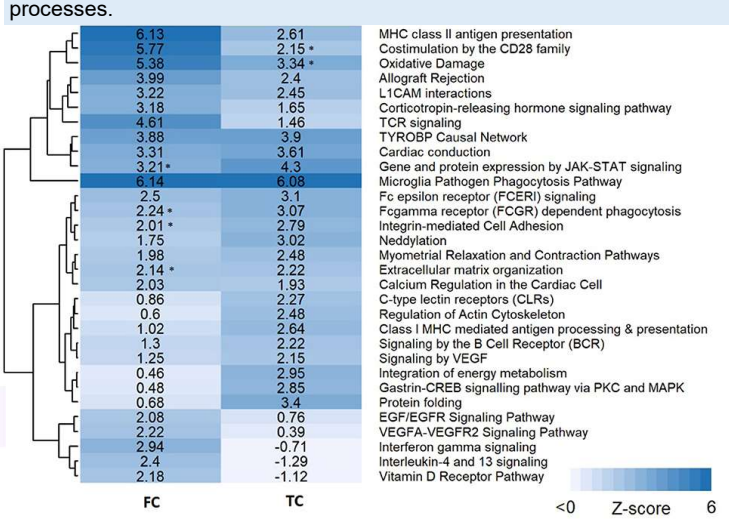
Study 2: WikiPathways network structure. Every interaction is represented as a node in the network with links to all participants. If the interaction is directed, the information about source and target nodes is added as an edge attribute. The nodes represented as small, red rounded rectangles are interactions, blue circles represent gene products and green diamonds embody metabolites. Interactions that share certain participants, such as GeneProduct 1, are brought close together in the resulting network even if they are from different pathways, such as Pathway 1 and 3.

Results: The integrative approach, using different datasets, pathway and network analysis methods to analyse transcriptomics datasets, revealed several downstream pathways of Rett syndrome and involved genes.

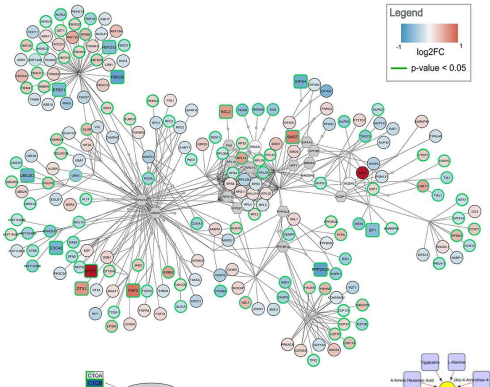
14 Genes of interest			GO processes of interest	Pathways of interest
HGNC symbol	Gene stable ID	Gene description	10 common elements in "Brain ", "Fibroblasts " and "Neuronal cells":	4 common elements in "Brain", "Neuronal cells" and "Fibroblasts":
ABAT	ENSG00000183044	4-aminobutyrate aminotransferase	negative regulation of inclusion body assembly(GO:0090084)	Apoptosis-related network due to altered Notch3 in ovarian cancer
ACAT2	ENSG00000120437	acetyl-CoA acetyltransferase 2	organ development(GO:0048513)	TGF-beta Receptor Signaling
CAPG	ENSG00000042493	Capping actin protein, gelsolin like	negative regulation of epithelial cell proliferation(GO:0050680)	Brain-Derived Neurotrophic Factor (BDNF) signaling pathway
CCL2	ENSG00000108691	C-C motif chemokine ligand 2	vasculogenesis(GO:0001570)	VEGFA_VEGFR2 Signaling Pathway
CDH2	ENSG00000170558	Cadherin 2	vascular endothelial growth factor receptor signaling pathway(GO:0048010)	
HSPA2	ENSG00000126803	Heat shock protein family A (Hsp70) member 2	lens development in camera-type eye(GO:0002088)	
LITAF	ENSG00000189067	Lipopolysaccharide induced TNF factor	blood vessel development(GO:0001568)	
MAP1B	ENSG00000131711	microtubule associated protein 1B	system development(GO:0048731)	
MEIS2	ENSG00000134138	Meis homeobox 2	blood vessel morphogenesis(GO:0048514)	
SERPING1	ENSG00000149131	Serpin family G member 1	response to ethanol(GO:0045471)	
SLC3A2	ENSG00000168003	solute carrier family 3 member 2		
SRSF11	ENSG00000116754	serine and arginine rich splicing factor 11		
TAGLN3	ENSG00000144834	transgelin 3		
YBX3	ENSG00000060138	Y-box binding protein 3		

Study 1: Table of differently expressed genes, common in different studies, samples and/or tissues in Rett patients vs. control group. Gene ontology (GO) and pathways commonly affected by these genes across the different studies and samples.

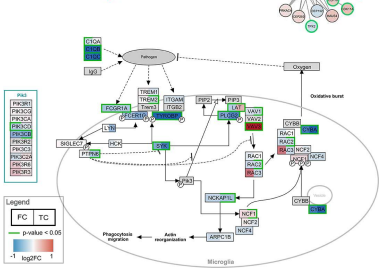
The different methods showed a clear overlap in ability to identify disease affected processes in inflammation, neuronal development, neuronal function, and translation. Pathway analysis was less effective to show affected processes in translation, which were identified clearly by gene ontology (data not shown) and active modules analysis. This is possibly due to a limited number of available pathways focussing on translation related processes.



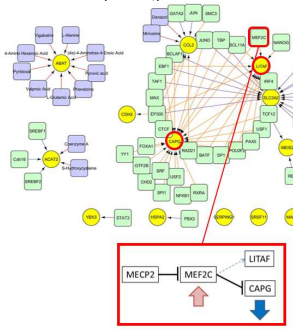
Study 2: Pathway analysis results for frontal and temporal cortex data. Pathways are clustered in this heatmap based on their Z-scores. Pathways with a high Z-score (>1.96) contain significantly more changed genes than expected and are considered pathways of interest. An asterisk next to the Z-score value indicates pathways with a significant Z-score (>1.96) but less than five changed genes.



Study 2: Top-ranked active module for frontal cortex data. The highest-ranked subnetwork contains 303 nodes and 568 edges. It contains 13 significantly changed genes (rounded rectangles) when applying the same cutoff as for enrichment analysis (absolute log2 fold change > 0.58). Other measured gene products are visualized as circular nodes. Blue fill color indicates down-regulation while red indicates up-regulation. The darker the color, the stronger the effect. Gray hexagons are gene products not measured in the data set. The very small, gray nodes represent interaction nodes. These were combined from 47 different pathways, with none of the pathways providing more than six interactions.



Study 2: Visualization of the frontal and temporal cortex gene expression on the Microglia Pathway Phagocytosis Pathway. In the left half of the gene boxes, the gene expression change in the frontal cortex is shown. In the right half of the gene boxes, the gene expression in the temporal cortex is shown. The blue colors represent down-regulation of the gene in Rett syndrome patients (negative log2 fold change), while the red shades visualize the up-regulated genes. The darker the color, the stronger the effect. Green borders indicate significance of the change (*p*-value < 0.05). Gray colored nodes are not annotated or measured in the dataset.



Study 1: Network extension for the DEGs (yellow) using data from ENCODE proximal and distant transcription factors (light green), drug targets and drugs (violet) for threshold absolute logFC > 1 gene list. MECP2WT inhibits expression of MEF2C, which inhibits expression of CAPG. If MECP2 is dysfunctional, CAPG was found to be mainly downregulated in the expression data. As MECP2 inhibition of MEF2C no longer works, MEF2C level increases (insignificant trend to upregulation found in data) and CAPG expression is inhibited. MEF2C is also a transcription factor for LITAF but the effect (stimulation or inhibition) is not clear from the data.

Conclusion: Although the details of identified pathways and active modules in the networks differed in each method used, the different methods showed a significant overlap in identification of pathways and processes which are clearly linked to the Rett syndrome phenotype in both studies.

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References
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