Long term regulation of glomerular gene expression in diabetic nephropathy

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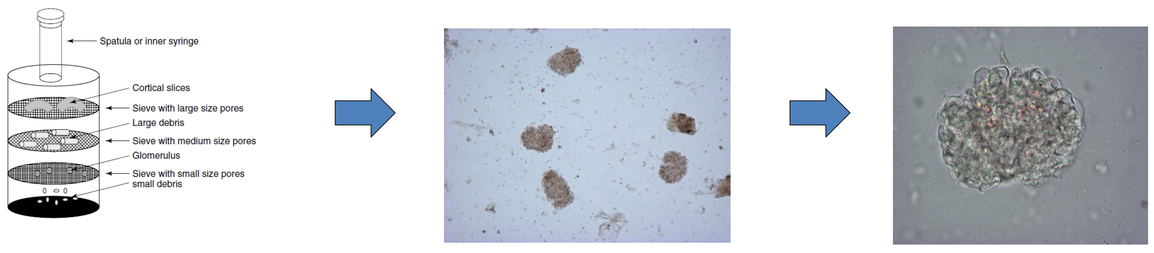
Background & Aim

Although Diabetic nephropathy (DN) is the most common cause for end-stage renal disease (ESRD) in Western societies, its pathogenesis still remains largely unclear.
The aim of this study was to identify candidate genes and gene clusters that could contribute to the development of diabetic kidney injury in type II diabetic mice.

Methods

1. Isolation of glomeruli from BTBR ob/ob and wildtype BTBR mice at the timepoints 4, 8, 16, 24 weeks.



BTBR ob/ob:
Homozygotous leptin deficient mice of the BTBR (black and tan, brachyuric) strand, develop a severe type II diabetes, but also diabetic kidney injury, with all the clinical and especially histologic features defining human DN

- ❖ 4 weeks: Proteinuria
- ❖ 8 weeks: Glomerular hypertrophy and accumulation of mesangial matrix
- ❖ 20 weeks: Glomerular lesions of human DN: thickening of glomerular basement membrane, expansion of mesangial matrix, nodular sclerosis, podocyte loss

Hudkins KL, …, Banas MC et al. , JASN 2010

2. Isolation of mRNA
3. *Affymetrix GeneChip Mouse Transcriptome Assay*
   => Identification of differentially expressed genes (DEGs)
4. Functional enrichment analysis
   => Identification of enriched biological processes (*Gene Ontology*) and pathways (*KEGG pathways*)

Results

Gene expression analysis of overall 65.956 gene loci (of these 10.692 coding genes).

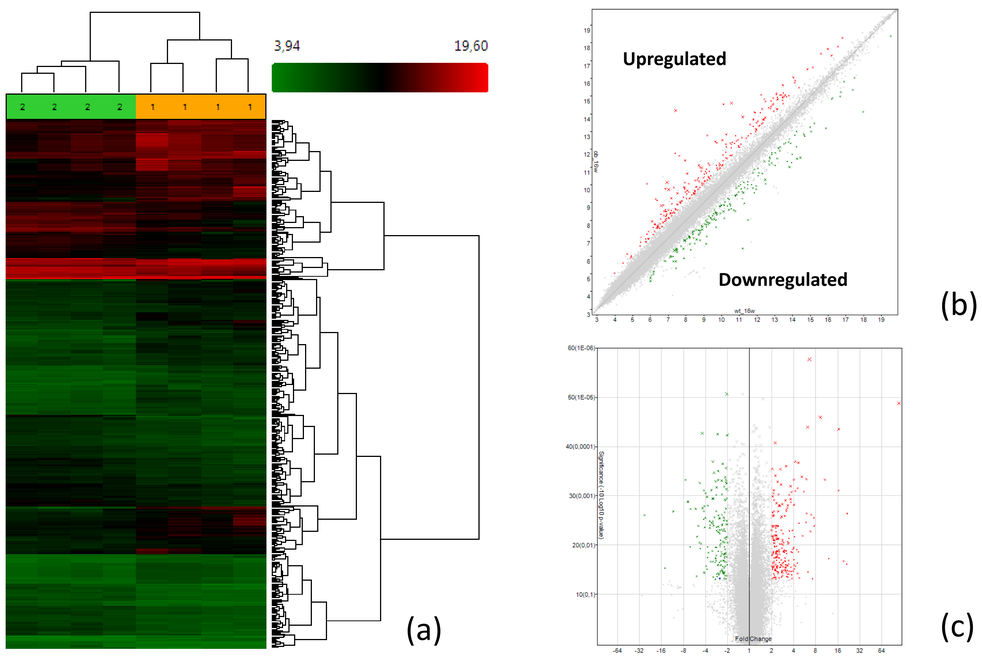


Fig. 1: Identification of DEGs from ob/ob vs. wildtype mice at an age of 16 weeks, using TAC analysis software.
(a) A heat map shows differences in the signal between the two probe sets.
(b) A scatter plot shows the fold changes of DEGs. DEGs with a fold change > 2 or < -2 and a p-value < 0,05 (one-way ANOVA) were defined as significantly altered. Genes with a fold change between -2 and 2 were excluded (grey area). Significantly upregulated DEGs are shown in red, downregulated DEGs in green.
(c) A volcano plot shows fold change of DEGs vs. significance calculated by one-way ANOVA. Again, upregulated DEGs in red, downregulated in green. Coloured DEGs were used for further functional enrichment analysis.

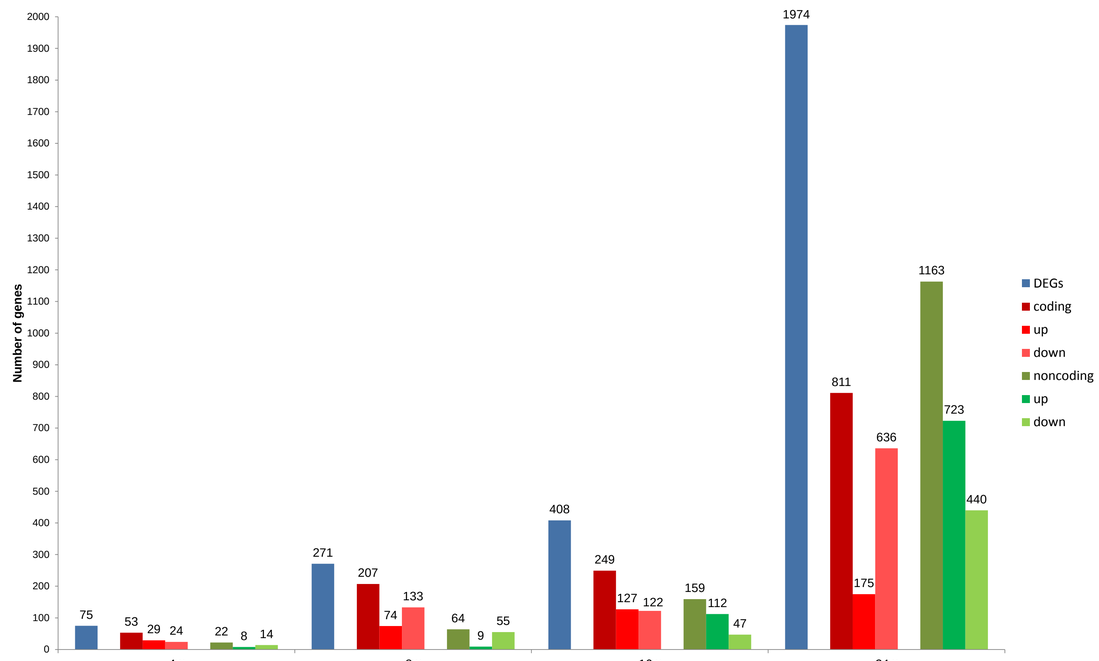


Fig.2.: Number of differentially expressed genes (DEGs, blue) in ob/ob mice vs. wildtype mice at 4, 8, 16 and 24 weeks. At each timepoint the distribution of coding (red), noncding (green) as well as up- and downregulated DEGs is shown.

Among the significantly differentially expressed genes over all 4 timepoints you find a lot of genes associated with glucose and lipid metabolism:

- **Renal glucose transporters** of the *solute carrier family* (Slc5a10, Slc2a5)
- ***3-Hydroxy-3-methylglutaryl-coenzymA-synthase 2 (Hmgcs2)*** (mitochondrial enzyme responsible for ketogenesis in hyperglycemia)
- Proteins linked to glykogenolysis and gluconeogenesis (**PKLR** = *pyruvat kinase isozymes L/R*, **PCK1** = *Phosphoenolpyruvate carboxykinase 1*)
- Members of Cytochrome P450 superfamily: Cyp21a2, Cyp21a1, Cyp27b1, Cyp24a1, Cyp2a4

Besides that you find genes potentially contributing to pathogenesis of DN, e.g.

Angiopoietin-like 4

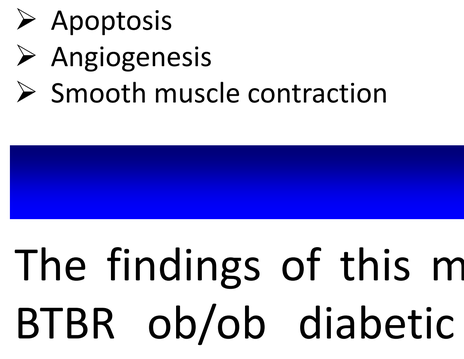


Fig. 3. The diagram shows *in vitro* results of real time qPCR for Angiopoietin-like-4 expression from whole kidney tissue in BTBR ob/ob mice of different age (x axis) compared to expression in wildtype mice of the same age.

GOID	GO term	p value	Number of involved genes
GO:0043436	Oxoacid metabolic process	3,7E-12	36
GO:0044281	Small molecule metabolic process	5,1E-12	55
GO:0019752	Carboxylic acid metabolic process	1,4E-11	35
GO:0032787	Monocarboxylic acid metabolic process	2,6E-11	29
GO:0006629	Lipid metabolic process	4,7E-11	43
GO:0006082	Organic acid metabolic process	5,5E-11	36
GO:0044710	Single-organism metabolic process	1,9E-10	81
GO:0006631	Fatty acid metabolic process	1,3E-9	22
GO:0044255	Cellular lipid metabolic process	9,9E-9	33
GO:1901700	Response to oxygen-containing compound	2,2E-8	46

(a)

KEGG pathway	p value	Number of involved genes
PPAR signaling pathway	1,6E-7	11
Fatty acid degradation	5,2E-6	8
Metabolic pathways	5,3E-5	36
Retinol metabolism	2,6E-4	8
Arachidonic acid metabolism	1,6E-3	7
Vascular smooth muscle contraction	2,2,E-3	8
Proximal tubule bicarbonate reclamation	3,6E-3	4
Inflammatory mediator regulation of TRP channels	9,0E-3	7

(b)

Table 1: Top 10 significantly enriched (a) Gene Ontology (GO) biological processes and (b) KEGG pathways for all upregulated DEGs, sorted ascending by p-value (one-way ANOVA < 0,01)

Additionally to the top 10 GO biological processes (BP), the significantly enriched BP include:

- ➢ Apoptosis
- ➢ Angiogenesis
- ➢ Smooth muscle contraction

Conclusion

The findings of this mRNA microarray from glomeruli of BTBR ob/ob diabetic mice can help to identify new candidate genes and gene clusters for the development of diabetic kidney injury in order to better understand its pathogenesis and to develop new therapeutic strategies.

Outlook

- ➢ Confirmation of results in cell culture experiments
- ➢ Confirmation of gene loci *in vivo*

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