Global characterization of gene expression regulation by clinical candidates liraglutide, elafibranor and obeticholic acid in a diet-induced obese mouse model with biopsy-confirmed NASH

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Introduction and Aim
The GLP-1 receptor agonist, liraglutide, the peroxisome proliferator-activated receptor (PPAR) a/S agonist, elafibranor, and the farnesoid X receptor agonist, obeticholic acid (OCA), are currently undergoing clinical investigation for the treatment of nonalcoholic steatohepatitis (NASH). The aim of the present study was to compare gene regulatory effects of liraglutide, elafibranor and OCA in a novel animal model of diet-induced obesity and biopsy-confirmed NASH – the Gubra DIO-NASH mouse. For comparison we also characterized gene regulation effect of diet regimes by comparing animal on Chow or DIO-NASH diet with animals on DIO-NASH diet reversed to Chow diet.

Methods
Male wildtype C57BL/6J and leptin-deficient Lepob/lepob mice (5 weeks of age) were fed a diet high in trans-fat, fructose and cholesterol for a total of 26 weeks for induction of NASH. Only biopsy-confirmed steatotic and fibrotic animals were included and stratified into DIO-NASH treatment groups and treated for 8 weeks with vehicle (PO, G0), liraglutide (0.2 mg/kg, SC, BID) or elafibranor (30 mg/kg, PO, G2) or reversed from DIO-NASH to Chow diet. At termination liver samples were collected and RNA was extracted subjected to RNAseq. RNAseq raw data was aligned to the mouse genome using STAR aligner, and the DESeq2 package for R was used to extract genes differentially expressed between treatment groups and controls.

Study Design

<table>
<thead>
<tr>
<th>Animal</th>
<th>Gender</th>
<th>Diet</th>
<th>Treatment</th>
<th>Administration</th>
<th>Daily Energy Intake (kcal)</th>
<th>Daily Protein Intake (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIO-NASH</td>
<td>Male</td>
<td>C57BL/6J</td>
<td>Vehicle</td>
<td>PO, G0</td>
<td>9 kcal</td>
<td>5 mg</td>
</tr>
<tr>
<td>DIO-NASH</td>
<td>Male</td>
<td>C57BL/6J</td>
<td>Elafibranor</td>
<td>SC, BID</td>
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<td>5 mg</td>
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<tr>
<td>DIO-NASH</td>
<td>Male</td>
<td>C57BL/6J</td>
<td>OCA</td>
<td>SC, BID</td>
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<tr>
<td>DIO-NASH</td>
<td>Male</td>
<td>C57BL/6J</td>
<td>Chow</td>
<td>SC, BID</td>
<td>9 kcal</td>
<td>5 mg</td>
</tr>
</tbody>
</table>

Results

Figure 1 Metabolic parameters by pharmacological treatment and Chow diet reversal in DIO-NASH animals.

Figure 2 Cluster analysis of treatment group gene expression profile. The most variable genes were subjected to principal component analysis and plots show the location of the first two principal components. Numbers in bracket indicate proportion of variance explained by component. All samples clustered according to treatment group with low intra-groups variation, indicating distinct and robust treatment specific regulation. Especially the elafibranor groups clustered far from the other groups. Small circles represent individual samples and large circles indicate the group center position.

Figure 3 Summary of differentially expressed genes at 5% FDR. A large overlap in differentially expressed genes was observed between compounds. However, many compound specific regulations could also be extracted. The reversal group affected the majority of genes differentially expressed between DIO-NASH and Chow.

Figure 4 NASH-centric pathway regulation summary. All genes differentially expressed versus control were mapped to a curated set of NASH-relevant pathways. Height of the bar indicate the proportion of genes in the pathway affected by the treatment. All treatments showed widespread perturbation of NASH related pathways. However, for most pathways OCA affected the highest number of genes followed by elafibranor and liraglutide. Chow and reversal groups also efficiently engaged the NASH pathways.

Figure 5 Monocyte recruitment response. Schematic overview of pathway with expression of selected genes in pathway. Data shown as mean±SEM. **: adj. p < 0.05, ***: adj. p < 0.005.

Figure 6 Stellate cell activation response. Schematic overview of pathway. Expression of selected genes in pathway. Data shown as mean±SEM. **: adj. p < 0.05, ***: adj. p < 0.005.

Conclusion
- Pharmacological intervention with liraglutide, elafibranor and OCA induced distinct and robust gene expression profile changes.
- Reversing the diet of DIO-NASH mice to Chow resulted in a gene expression profile nearly identical to that of healthy Chow mice.
- Gene expression changes induced by elafibranor was clearly distinct from other groups on principal component analysis.
- All compounds and the diet reversal caused pronounced regulation of NASH-relevant signaling pathways.
- At single-gene level clear reduction of prototypical NASH related to inflammation and fibrosis genes could be identified after all treatments.
- Despite no change in Col1a1 protein level, robust reduction of Col1a1 mRNA, indicating fibrosis inhibition, could be detected.