Role of the Organic Solute Transporter OSTα-OSTβ in Intestinal Bile Acid Transport and Homeostasis

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Properties of Mouse Ostα-Ostβ

• Expressed in small intestine, cecum, colon, kidney

• Tissue expression is similar to Asbt, with highest levels in ileum

• Expressed on basolateral membrane of the ileal enterocyte

• Efflux substrate specificity includes the major species of bile acids

• Expression of both genes is positively regulated by bile acids via FXR
Expression of Ostα and Ostβ mRNA and Protein in Wild Type and Ostα Null Mice

Ostα and Ostβ mRNA Expression in Wild Type and Ostα Null Mice

Males
Females

Ileum

Ostα

+/
-/
+/
-/

Actin

Kid S1 S2 S3 S4 S5 Ce Pc Dc

Ostα mRNA Expression (arbitrary units)

Ostβ mRNA Expression (arbitrary units)
Phenotypic Comparison of Wild Type and Ostα Null Mice

- Ostα KO pups show a growth deficit that is ameliorated after weaning

- Adult WT and KO mice were identical with regard to:
  - Prenatal Survival
  - Postnatal Survival
  - Gross Appearance
  - Behavior
Phenotypic Comparison of Wild Type and Ostα Null Mice

- Ostα KO mice have longer and heavier small intestines

<table>
<thead>
<tr>
<th>Genotype</th>
<th>WT</th>
<th>Ostα KO</th>
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<tbody>
<tr>
<td>+/+</td>
<td></td>
<td></td>
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<tr>
<td>-/-</td>
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</table>

Intestinal Length (cm)

Intestinal Length (mm/100 g BW)

Segment Wet Weight (mg/cm)

Small Intestinal Segment

- Proximal
- Distal

$P = 0.006$

$P < 0.0001$

$P < 0.05$
Ileal Histology of Wild Type and Ostα Null Mice

Wild Type

Ostα KO
Taurocholate Transport in Everted Gut Sacs

Males

Females

Taurocholate Transport (M->S) (nmol/30 min/g tissue)

Jejunum

Ileum

Jejunum

Ileum

Ostα+/

Ostα−/

Ostα+/ Mrp3−/

Ostα−/ Mrp3−/

Asbt−/

Taurocholate Transport (M->S) (nmol/30 min/g tissue)

Jejunum

Ileum

Ostα+/

Ostα−/

Ostα+/ Mrp3−/

Ostα−/ Mrp3−/

Asbt−/
Taurocholate Transport in Everted Ileal Gut Sacs

Taurocholate Transport (M→S) (nmol/30 min/ g tissue)

Males
Females

<table>
<thead>
<tr>
<th></th>
<th>Ostα−/−</th>
<th>Ostα−/− Mrp3−/−</th>
<th>Asbt−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical</td>
<td>Mrp3</td>
<td>Ost</td>
<td>TC</td>
</tr>
<tr>
<td>Basolateral</td>
<td>TC</td>
<td>Asbt</td>
<td>Mrp3</td>
</tr>
</tbody>
</table>

PAD
Bile Acid Pool Size and Fecal Bile Acid Excretion in Wild Type and Ostα Null Mice

**Pool Size**

<table>
<thead>
<tr>
<th>Bile Acid Pool Size (µmol/100 g bw)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tauroursodeoxycholate</td>
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<tr>
<td>Taurodeoxycholate</td>
<td></td>
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<tr>
<td>Tauro-β-murichol.</td>
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<tr>
<td>Taurocholate</td>
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**Fecal Excretion**

<table>
<thead>
<tr>
<th>Fecal Bile Acid Excretion (mol/day per 100 g bw)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ostα⁺⁺/⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostα⁻⁻/⁻</td>
<td></td>
<td></td>
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</tbody>
</table>
Model for Altered Bile Acid Homeostasis in Ostα Null Mice

**Wild Type**

Ileal Enterocyte

- ASBT
- Bile acids

- Fxr
- FGF 15

- FGFR4

- Cyp7a1 gene

Hepatocyte

- FGF15

**Asbt KO**

- ASBT
- Fxr
- FGF 15

- Cyp7a1 gene

**Ostα KO**

- ASBT
- Fxr
- FGF 15

- Cyp7a1 gene
Gene Expression in Asbt and Ostα Null Mice

Ileal Enterocyte
- Bile acids → ASBT → FGF15
- FGF15 → FGFR4 → Cyp7a1 gene

Hepatocyte
- FGF15

mRNA Expression (% of wild type)

Ileum
- FGF15
- Genotype: WT, KO

Liver
- Cyp7a1
- Genotype: WT, KO
Fecal Bile Acid Excretion in Wild Type and Ostα Null Mice Fed a 0.2% Cholic Acid-Containing Diet
1) Ostα-Ostβ is a major intestinal basolateral bile acid transporter and is essential for bile acid homeostasis.

2) Mrp3 is not essential for intestinal absorption of bile acids in mice, but can serve a secondary role particularly in females.

3) Inhibition of basolateral versus apical intestinal absorption alters the classical feedback regulation of hepatic bile acid synthesis and may significantly alter lipid metabolism.
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  Lou Craddock

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  Gary Kruh
  Marty Belinsky

Intestinal Gene Expression in Wild Type and Ostα Null Mice

Asbt mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Asbt mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Ilbp mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Ilbp mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Shp mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Shp mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Mrp3 mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Mrp3 mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Shp mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Shp mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Mrp3 mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Mrp3 mRNA Expression (normalized to cyclophilin)

Ostα+/+  Ostα−/−

Intestinal Gene Expression in Wild Type and Ostα Null Mice
Intestinal Gene Expression in Wild Type and Ostα Null Mice

Mrp2 mRNA Expression (normalized to cyclophilin)

Ostα⁺/+  Ostα⁻⁻  Mrp2

Proximal Distal

Mrp4 mRNA Expression (normalized to cyclophilin)

Ostα⁺/+  Ostα⁻⁻  Mrp4

Proximal Distal

Bcrp mRNA Expression (normalized to cyclophilin)

Ostα⁺/+  Ostα⁻⁻  Bcrp

Proximal Distal

Mrp3 mRNA Expression (normalized to cyclophilin)

Ostα⁺/+  Ostα⁻⁻  Mrp3

Proximal Distal
Bile Acid Transport by Intestinal Epithelial Cells

Jejunum

Ileum

Intestinal Lumen

Colon

Cholic $\rightarrow$ Deoxycholic

Portal Circulation