Aim
Our aim is to find the molecular cause of intestinal malrotation and map the genes needed for normal development of the intestines.

- We want to be able to give information on cause, recurrence risk, associated genetic diseases and also to decrease the feeling of guilt that can be experienced by parents when a child is born with a malformation. Increased knowledge on inheritance can lead to earlier diagnosis, which will lead to less suffering.
- This can contribute to an understanding in what genes are important in the normal embryonic development of the intestines and can be of importance in other fields.
- Further it can change the routines for genetic investigation in this patient group.

Methods and Results
DNA from n=47 patients with symptomatic intestinal malrotation was analyzed with array comparative genomic hybridization (aCGH), a method to find copy number variants (CNVs), comparing the patients DNA to a reference DNA. We used a custom high-resolution oligonucleotide aCGH with a 2 × 400K oligonucleotide probe design targeting 1,989 genes, including all genes in the cilia proteome and known malformation syndromes.

We identified six rare CNVs in five patients. Five CNVs involved known syndrome loci: 7q11.23 microduplication, 16p13.11 microduplication, 18q terminal deletion, HDAC8 (Cornelia de Lange syndrome type 5 and FOXF1) and also one intragenic deletion in GALNT14, not previously implicated in human disease. All patients had other malformations except for patient 1.

### Table 1. Rare CNVs identified in five patients with intestinal malrotation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Array result</th>
<th>Inheritance</th>
<th>Coordinates</th>
<th>Affected genes/loci</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heterozygous deletion</td>
<td>Paternal</td>
<td>2q23.1</td>
<td>123,336</td>
<td>GALNT14</td>
</tr>
<tr>
<td>2</td>
<td>Duplication</td>
<td>De novo</td>
<td>7q11.23</td>
<td>1,007,454</td>
<td>7q11.23 microduplication</td>
</tr>
<tr>
<td>3</td>
<td>Heterozygous deletion</td>
<td>NI</td>
<td>16p22.1</td>
<td>13,158,366</td>
<td>16q deletion syndrome</td>
</tr>
<tr>
<td>4</td>
<td>Heterozygous deletion</td>
<td>De novo</td>
<td>9q31.1</td>
<td>487,626</td>
<td>HDAC8 (Cornelia de Lange type 5)</td>
</tr>
<tr>
<td>5</td>
<td>Duplication</td>
<td>De novo</td>
<td>6p24</td>
<td>1,469,327</td>
<td>FOXF1</td>
</tr>
</tbody>
</table>

Ni: No information; VOSD: Variant of uncertain significance; Breakpoints sequenced: exact breakpoints

### Intestinal development
During normal fetal development the intestines rotate 270 degrees counterclockwise to end up in its normal position. A disturbance in this process leads to intestinal malrotation. Intestinal malrotation stands for an incomplete rotation and fixation of the midgut during fetal development.

### Future perspectives
Whole Genome Sequencing
The literature and our previous studies have given us a reason to hypothesise that there are other genetic alterations to find in patients with intestinal malrotation. With whole genome sequencing (WGS) we can find smaller and different kinds of alterations (single-nucleotide variations, insertions and deletions, translocations and large structural variants).

This is an ongoing part of the project where we perform WGG on patients with isolated intestinal malrotation (no other malformations). Panels with candidate genes (from the literature and our findings) will initially be used, followed by more detailed analysis.

### Zebrafish model
Findings will be verified with Sanger sequencing and used in a zebrafish model. A zebrafish model is an appropriate model for this study as it exhibits left-right asymmetry of the intestines, it has a fast reproductive cycle and it covers a large part of the human genome, especially during fetal development.

Conclusions
In the present study, we identified rare CNVs contributing pathogenic or potentially pathogenic alleles in five patients with syndromic intestinal malrotation, suggesting that CNV screening is indicated in intestinal malrotation with associated malformations or neurological involvements. In addition, we identified intestinal malrotation in two known syndromes (Cornelia de Lange type 5 and 18q terminal deletion syndrome) that has not previously been associated with gastrointestinal malformations.