A Novel Role of CC2D1A in Human Heterotaxy and Ciliary Dysfunction


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Introduction
Heterotaxy, also known as situs ambiguous, is a class of human congenital disorders that are characterized by a failure to establish normal left-right (L–R) asymmetry and by the misplacement of one or more organs during embryonic development. The estimated incidence at birth is around 1 in 10,000-5. Cardiovascular malformations are commonly associated with heterotaxy and account for approximately 3% of all congenital heart defects4-5. The genetic causes of human heterotaxy are highly heterogeneous.

Methods
We performed exome sequencing in a cohort of 26 probands with heterotaxy. Burden analysis was performed by SKAT to identify genes with statistical significance compared to local controls and repeated with data from Exome Aggregation Consortium Browser (ExAC). Transcription activator-like effector nuclease (TALEN) was used to generate somatic loss-of-function mutants in a zebrafish model. Visceral organ development was examined by WISH for ccnt1 and foxa3. Whole-mount immunostaining of acetylated α-tubulin was performed for cilia conformation.

Results
We identified a significant enrichment of novel rare damaging mutations in the CC2D1A gene (Figure 1 and Table 1). Seven occurrences of CC2D1A mutations were found to affect four highly conserved amino acid residues of the protein (Figure 2). We further evaluated the novel roles of CC2D1A by functional analyses in the TALEN-mediated zebrafish knock-out models and identified heterotaxy phenotypes of the cardiovascular and gastrointestinal systems in both somatic and germline mutants (Figure 3). Defective conformation in cc2d1a mutant embryos was shown along the spinal canal and pronephric duct via whole-mount immunostaining of acetylated α-tubulin (Figure 4).

Table 1. Mutation burden of CC2D1A in cases in four control groups.

<table>
<thead>
<tr>
<th>Sample groups</th>
<th>Sample size</th>
<th>Samples with rare damaging missense mutations in CC2D1A</th>
<th>Frequency</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>SKAT p value</th>
<th>Corrected p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>26</td>
<td>6</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal Control</td>
<td>130</td>
<td>2</td>
<td>0.02</td>
<td>19.2</td>
<td>3.6, 101.8</td>
<td>3.34E-06</td>
<td>3.79E-02</td>
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<tr>
<td>ESP0500 Control</td>
<td>6525</td>
<td>74</td>
<td>0.01</td>
<td>26.1</td>
<td>10.1, 67.0</td>
<td>3.81E-08</td>
<td>7.16E-04</td>
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<tr>
<td>ExAC control</td>
<td>61486</td>
<td>936</td>
<td>0.02</td>
<td>19.4</td>
<td>7.8, 48.4</td>
<td>1.97E-07</td>
<td>3.70E-03</td>
</tr>
</tbody>
</table>

The odds ratio refers to the ratio between the odds of cases with mutations and the odds of controls with mutations.

Acknowledgement
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References