HKUMed discovers a novel gene in causing the rare disease “heterotaxy syndrome”

Professor Cheung Yiu-fai, Dr Brian Chung Hon-yin, Dr Christopher Mak Chun-yu & Dr Yeung Kit-san
Heterotaxy syndrome (includes right & left atrial isomerism)

- In human bodies, the left side & right side are not identical.
- Human heart is asymmetrical.
- Heterotaxy syndrome is a group of rare, complex condition that involves the abnormal arrangement of internal organs, including the heart, on the wrong side of the body.
- The estimated incidence of heterotaxy is around 1 per 10,000 births1^-3. If abortions & stillbirths are included, it accounts for 0.03% to 1.1%4.

References:

Treating heterotaxy is difficult & complicated

- Approximately 90% of heterotaxy patients have complex congenital heart defects
- Multiple body systems can be affected, including the heart, lungs, spleen, stomach, liver and intestines
- Management by surgical operation is associated with multi-systemic complications

2. Shiraishi I et al. Circ J. 2012;76(9):2066-75
HKUMed Paediatric Cardiology Division has longitudinally followed up patients with right atrial isomerism & reported on their clinical outcomes.

Kaplan-Meier survival estimates for presented 116 infants and children determined to have right atrial isomerism between January 1980 and December 2000 in Hong Kong.


Right isomerism leads to significant mortality.

現時病人的存活率有明顯改善
Improved survival rate nowadays

右心房異位症會大幅提高死亡率

Left isomerism leads to significant mortality.
Cilia defects may cause heterotaxy syndrome

- Growing evidence that abnormal function of cilia can result in congenital heart disease, & errors in establishing left-right asymmetry

- Multiple genes involved (genetic heterogeneity)


Image: American Heart Association Scientific Statement, Genetic Basis for Congenital Heart Disease: Revisited. ¹
• Cilia defects may cause abnormal flow of signals that guide the left-right axis formation, resulting in heterotaxy\(^1\)


Image: Shiraishi I et al. Circ J. 2012;76(9):2066-75
Research gap & aims of study

The genetic causes of Hong Kong patients with heterotaxy is unknown, therefore we:

• perform exome sequencing to identify the disease-causing mutations; and

• investigate the impact of mutations on left-right axis development & cilia defects.
Whole exome sequencing on 26 heterotaxy patients

- Exome refers to the collection of coding regions of all the genes
- Human exome accounts for 1% of human genome
- About 85% of pathogenic mutations can be found in human exome

NO pathogenic mutations can be identified in known genes associated with heterotaxy in our patients

⇒ Next step is to identify new genes that have not been associated with heterotaxy

在我們的異位綜合症病人中找不到任何已知的遺傳成因
⇒ 下一步，我們嘗試找出過去從未被發現與異位綜合症相關的新遺傳成因
Significant enrichment of \textit{CC2D1A} damaging variants in patients compared to controls.

<table>
<thead>
<tr>
<th>Sample groups</th>
<th>Sample size</th>
<th>Samples with rare damaging missense mutations in \textit{CC2D1A}</th>
<th>Frequency</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>SKAT p value</th>
<th>Corrected p value</th>
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<tbody>
<tr>
<td>Case</td>
<td>26</td>
<td>6</td>
<td>0.23</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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<td>Internal Control</td>
<td>130</td>
<td>2</td>
<td>0.02</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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<td>ESP6500 Control</td>
<td>6525</td>
<td>74</td>
<td>0.01</td>
<td>19.4</td>
<td>7.8, 48.4</td>
<td>1.97E-07</td>
<td>3.70E-03</td>
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<tr>
<td>ExAC control</td>
<td>61486</td>
<td>936</td>
<td>0.02</td>
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The odds ratio refers to the ratio between the odds of cases with mutations and the odds of controls with mutations.
**CC2D1A 已知的功用**
Known facts about **CC2D1A**

**CC2D1A: Coiled-coil and C2 domain containing 1A**
- Located on chromosome 19, contains 31 exons
- Gene size: 24,731 base pairs; protein size: 951 amino acids; contains 4 tandem repeat of DM14 + 1 C2 domains
- Role in left-right axis formation and cilia function have never been reported

[Diagram of Human CC2D1A]
與CC2D1A有關的表徵
Phenotypes associated with CC2D1A

老鼠
• 基因剔除CC2D1A後會令老鼠出生後出現呼吸困難，立即死亡

人類
• 牽涉第14至16個外顯子的同型接合缺失導致智力障礙
• 19p13.2-p13.12的異型接合缺失(包含另外6個基因)導致發展遲緩

Mouse:
• CC2D1A knockout mouse is lethal, with cyanosis & breathing difficulties

Human:
• Homozygous deletion involving exons 14 to 16 resulted in non-syndromic intellectual disability
• Heterozygous deletion in 19p13.2-p13.12 (includes 6 other genes) resulted in developmental delay

未有醫學文獻記錄CC2D1A與左右軸排列和纖毛功能的關聯

Role of CC2D1A in left-right axis formation and cilia function have never been reported

Advantages of using zebrafish for research\textsuperscript{1,2}:

- Transparent during development
- High fertility & easy to house in a large quality $\Rightarrow$ large scale screening is possible
- Zebrafish heart & human heart undergo similar morphogenetic processes
- Develop from fertilised egg to embryo in 24 hours post fertilisation, with established contracting heart tube

CC2D1A in human and zebrafish is highly conserved.

**Human**
- CC2D1A
  - P192L
  - Q506R

**Zebrafish**
- cc2d1a
  - P559
  - G808

**Equivalences**
- $CC2D1A^{P532L} = cc2d1a^{P559L}$
- $CC2D1A^{G781V} = cc2d1a^{G808V}$
Creating a knock-out *cc2d1a* zebrafish model by genome editing

**Diagram:**
- Human *CC2D1A*:
  - DM14
  - Q506R
  - P532L
  - C2
  - G781E
  - G781V
- Zebrafish *cc2d1a*:
  - DM14
  - P559
  - G808

**Text:**
- Cutting DNA of zebrafish *cc2d1a* by gene editing (TALEN), leading to an abnormal protein that lost its functions
cc2d1a 的異變導致斑馬魚心臟和消化系統異常
Zebrafish with cc2d1a mutation showed heart & digestive system disarrangement

Injections of wild type cc2d1a to the edited fertilised egg can rescue the abnormal organ arrangement
Mutations in *cc2d1a* were associated with cilia defects

**A** 48 hpf
- **Control**
  - Normal (86/91)
- *cc2d1a TALEN*  \( p < 0.0001 \)
  - Normal (56/93)
  - Mildly curved (25/93)
  - Moderate curved (12/93)

**B** 72 hpf
- **Control**
  - Normal (92/92)
- *cc2d1a TALEN*  \( p < 0.0001 \)
  - Normal (68/102)
  - Single (29/102)
  - Supernumerary (5/102)

**C** 18 hpf
- **Control**
  - Normal (66/69)
- *cc2d1a TALEN*  \( p < 0.0001 \)
  - Normal (45/65)
  - Mirrored (8/65)
  - Bilateral (12/65)

**spaw**

耳石發展
Otolith development

spaw的表達（其中一個左右軸排列所需的生物信號）
*spaw* expression
**cc2d1a**的異變與纖毛缺陷相關

Mutations in *cc2d1a* were associated with cilia defects

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注射沒有異變的*cc2d1a*到基因編輯的胚胎可以令纖毛回復正常

Injections of wild type *cc2d1a* to the edited fertilized egg can rescue the cilia defects
Mutations in *cc2d1a* were associated with fewer & shorter cilia in zebrafish cells.

**cc2d1a**的異變令斑馬魚細胞的纖毛減少及變短
Summary

- **CC2D1A** – Novel gene discovery in causing heterotaxy
  - Found in 6 out of 26 patients with heterotaxy (19%)
  - Implicated in non-syndromic congenital heart disease
  - The disease mechanism of CC2D1A is possibly due to cilia defects
Preimplantation genetic diagnosis of CC2D1A in families with family history is possible

Prenatal diagnosis is possible, but reduced penetrance & variable expressivity are caveats

It is important to study whether cilia defects affects other body systems, especially the respiratory system

According to the 2018 updated scientific statement on congenital heart disease from the American Heart Association, respiratory complications are one of the most important modulators of post-operative outcome. Therefore, further studies are required to evaluate the possibility of airway ciliary defects in this group of patients and hence tailored respiratory treatment is possible¹

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