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港大醫學院發現構成罕見疾病異位綜合症的全新遺傳成因

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

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異位綜合症 （包括右心房異位和左心房異位）
Heterotaxy syndrome (includes right & left atrial isomerism)

右心房異位　　左心房異位

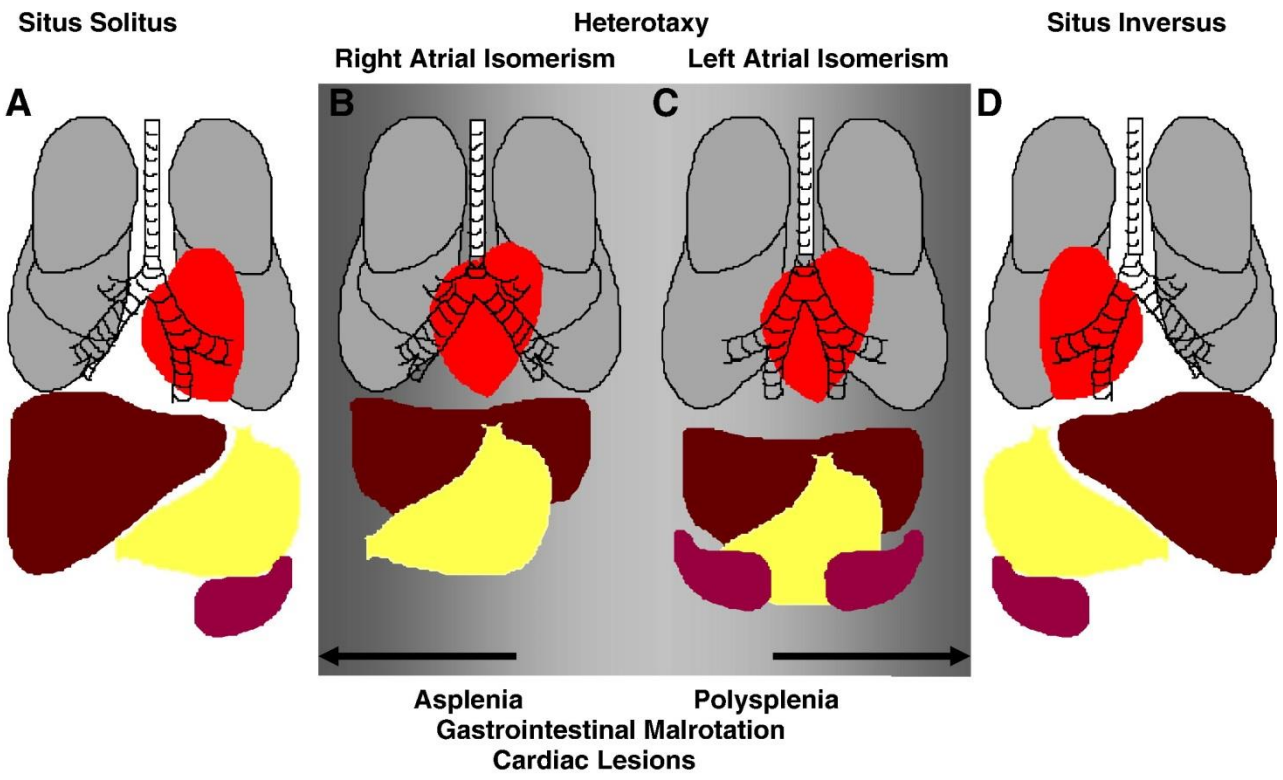

Image: Tashjian et al. J Pediatr Surg. 2007 Mar;42(3):528-31

- 在人類的身體，左邊和右邊並不是一樣的；人類心臟並不是對稱的
- 異位綜合症是一種先天性的疾病，特徵包括一個或多個器官的排列組合出現問題
- 發生率大約為每一萬名初生嬰兒中會有一個[1-3]；若果包括流產的胚胎，發生率大概是0.03%至1.1%[4]

- 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 births[1-3]. If abortions & stillbirths are included, it accounts for 0.03% to 1.1% of fetuses[4]

1. Maclean et al. Clinical genetics. 2004 Jun;65(6):441-57
2. Shapiro et al. Chest. 2014 Nov 1;146(5):1176-86
3. Lin et al. American journal of medical genetics Part A. 2014 Oct;164(10):2581-91
4. Bartram et al. Biol Neonate 2005 Aug;88:278–290

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治療異位綜合症是相當困難
Treating heterotaxy is difficult & complicated

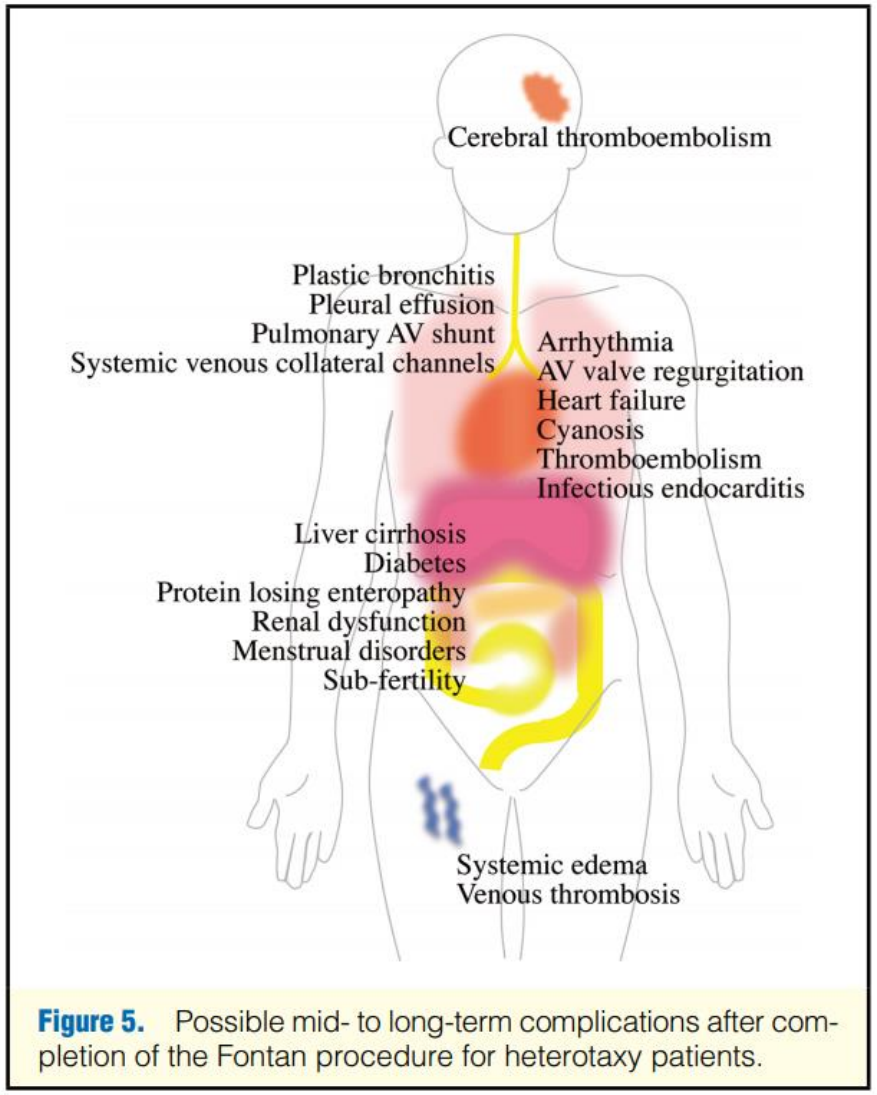

Figure 5. Possible mid- to long-term complications after completion of the Fontan procedure for heterotaxy patients.

Image: Shiraishi I et al. Circ J. 2012;76(9):2066-75

- 大約90%的異位綜合症患者患有先天性心臟病[1]
- 多個器官都可能受到影響，包括心臟、肺、脾、胃、肝和腸[2]
- 外科手術治療有機會引致身體多個系統有併發症[2]
- Approximately 90% of heterotaxy patients have complex congenital heart defects[1]
- Multiple body systems can be affected, including the heart, lungs, spleen, stomach, liver and intestines[2]
- Management by surgical operation is associated with multi-systemic complications[2]

1. Lin et al. Genetics in Medicine. 2000 May;2(3):157-72
2. Shiraishi I et al. Circ J. 2012;76(9):2066-75

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港大醫學院兒童心臟科長期照料雙右心房異位的病人
HKUMed Paediatric Cardiology Division has longitudinally followed up patients with right atrial isomerism & reported on their clinical outcomes

BMJ Journals **Heart**

Congenital heart disease

Outcome of infants with right atrial isomerism: is prognosis better with normal pulmonary venous drainage? FREE

Y F Cheung[1], V Y W Cheng[1], A K T Chau[1], C S W Chiu[2], T C Yung[1], M P Leung[1]

Author affiliations

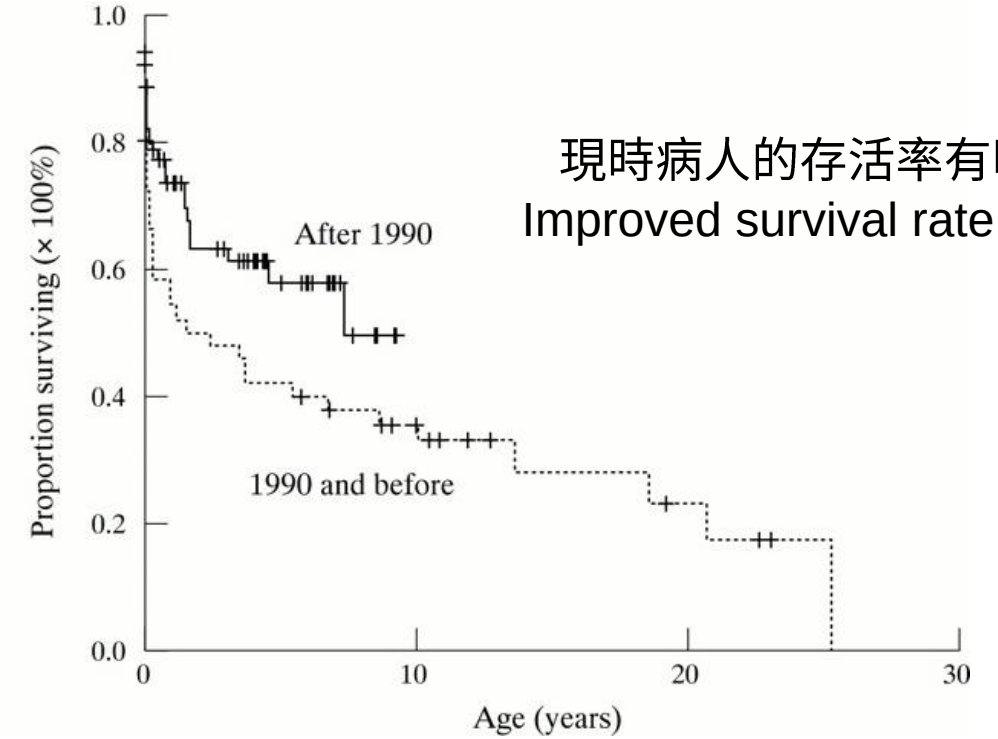
1. Division of Paediatric Cardiology, Department of Paediatrics, Grantham Hospital, The University of Hong Kong, Hong Kong, People's Republic of China

AHJ American Heart Journal

Electrophysiology

Cardiac rhythm and symptomatic arrhythmia in right atrial isomerism

Yiu-fai Cheung, MBBS, Vinson Yan-wah Cheng, MBChB, Tak-cheung Yung, MBBS, and Adolphus Kai-tung Chau, MBBS *Hong Kong, People's Republic of China*



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

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[1]

1. Cheung YF et al. Heart. 2002 Feb;87(2):146-52
2. Cheung YF et al. Am Heart J. 2002 Jul;144(1):159-64

右心房異位症會大幅提高死亡率
Right isomerism leads to significant mortality

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纖毛缺陷有機會構成異位綜合症
Cilia defects may cause heterotaxy syndrome

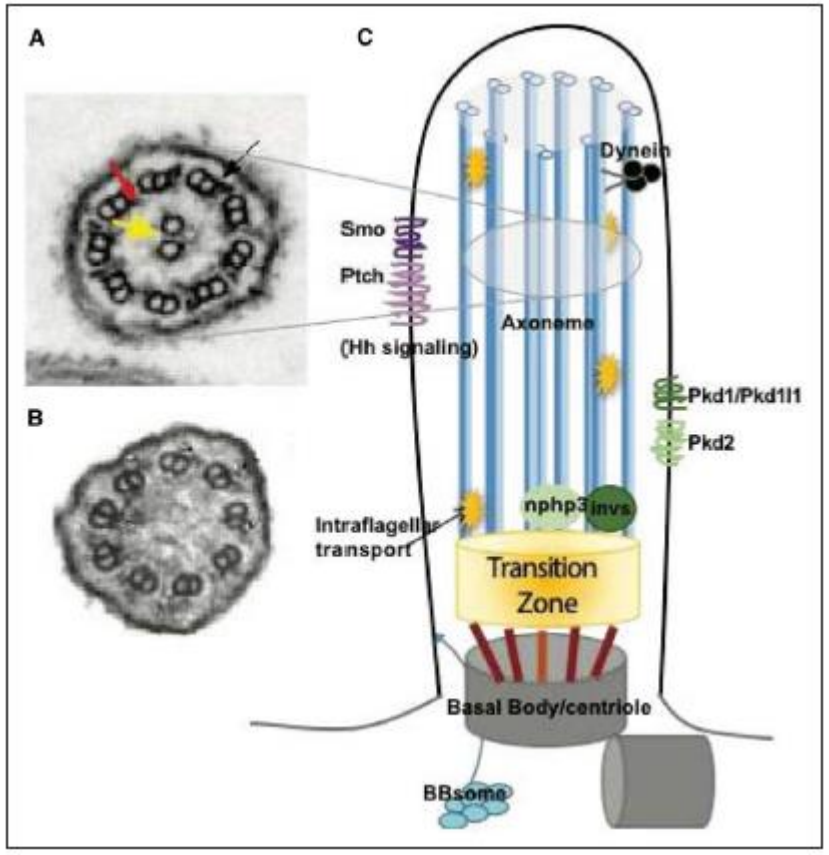

Image: American Heart Association Scientific Statement, Genetic Basis for Congenital Heart Disease: Revisited. [1]

- 越來越多證據顯示纖毛功能異常可以導致先天性心臟病，亦會導致左右不對稱[1]
- 涉及多個基因（遺傳異質性）

- Growing evidence that abnormal function of cilia can result in congenital heart disease, & errors in establishing left-right asymmetry[1]
- Multiple genes involved (genetic heterogeneity)

1. Pierpont ME et al. Circulation. 2018 Nov 20;138(21):e653-e711

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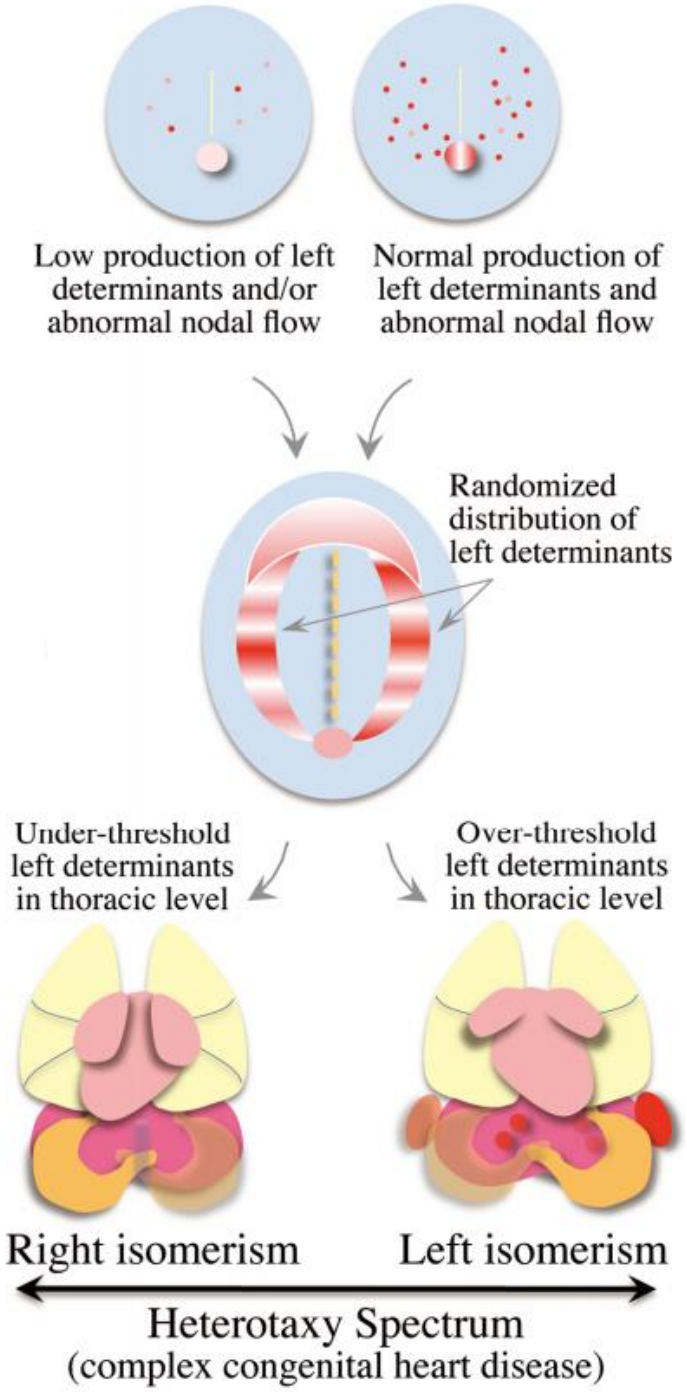
異位綜合症 與 纖毛缺陷
Heterotaxy and Cilia Defects



- 器官要形成正確的左右軸排列，需要經相關的生物信號所引導，但纖毛缺陷會錯誤傳遞這些信號，最後導致異位綜合症[1]

- Cilia defects may cause abnormal flow of signals that guide the left-right axis formation, resulting in heterotaxy[1]

1. Shiraishi I et al. Circ J. 2012;76(9):2066-75

Image: Shiraishi I et al. Circ J. 2012;76(9):2066-75

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研究缺口與目的
Research gap & aims of study

構成本地異位綜合症的遺傳成因未明，因此我們：

- 為病人進行外顯子測序來找出相關的基因異變，及
- 研究這些基因異變對左右軸排列和纖毛缺陷所帶來的影響

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.

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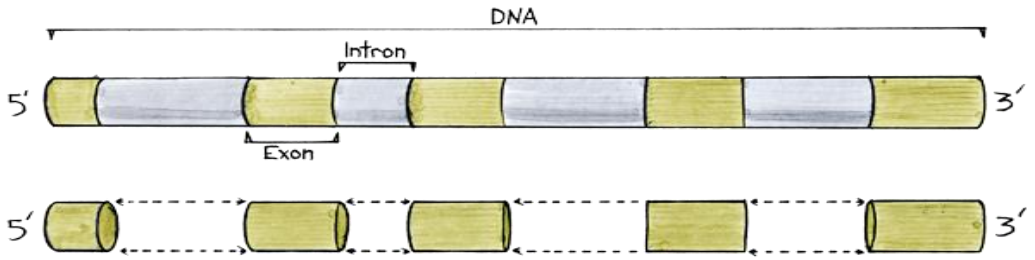
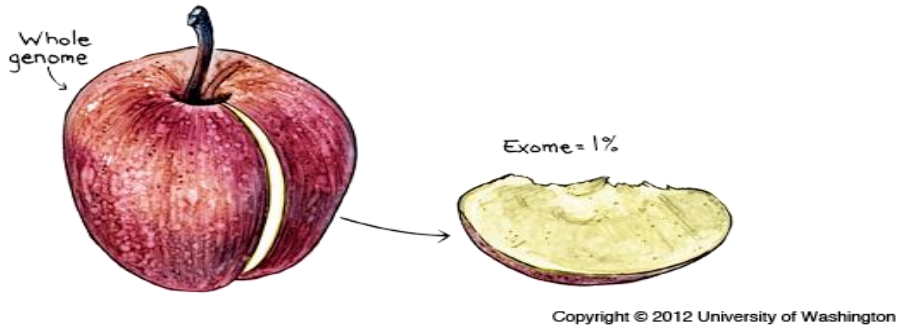
為26位異位綜合症病人進行全外顯子測序
Whole exome sequencing on 26 heterotaxy patients

- 所有可以轉譯成蛋白質的DNA稱之為「外顯子 (exome)」
- 外顯子佔了人類基因體大概1%
- 大約有85%由基因異變所引起的疾病發生在外顯子區域



- 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



在我們的異位綜合症病人中**找不到**任何已知的遺傳成因

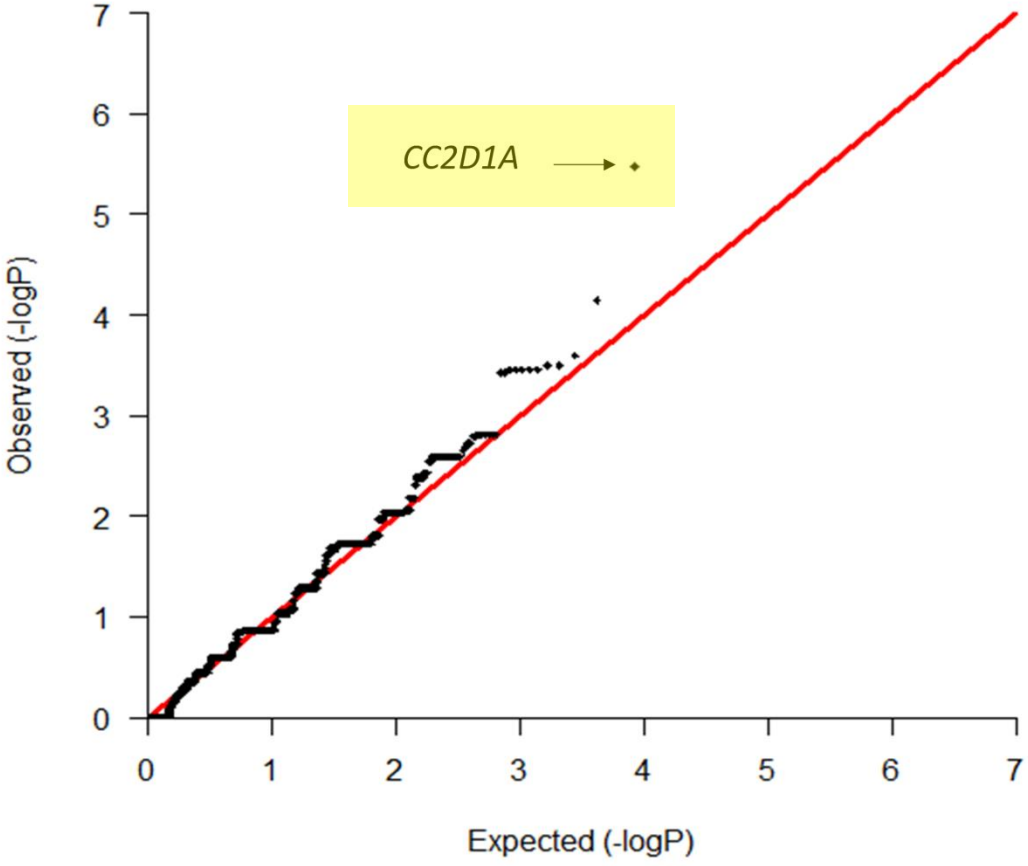
➔ 下一步，我們嘗試找出過往從未被發現與異位綜合症相關的新遺傳成因

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

與對照組相比，在病人身上找到更多具破壞性的*CC2D1A*變異
Significant enrichment of *CC2D1A* damaging variants in patients compared to controls

Gene Burden Analysis in heterotaxy



Mutation burden of *CC2D1A* in patients vs. three control groups

Sample groups	Sample size	Samples with rare damaging missense mutations in *CC2D1A*	Frequency	Odds ratio	95% Confidence interval	SKAT p value	Corrected p value
Case	26	6	0.23				
Internal Control	130	2	0.02	19.2	3.6, 101.8	3.34E-06	3.79E-02
ESP6500 Control	6525	74	0.01	26.1	10.1, 67.0	3.81E-08	7.16E-04
ExAC control	61486	936	0.02	19.4	7.8, 48.4	1.97E-07	3.70E-03

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

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CC2D1A 已知的功用
Known facts about *CC2D1A*

***CC2D1A*: Coiled-coil and C2 domain containing 1A**

- 位於第19對染色體，有31個外顯子
- 基因有24,731個鹼基對；蛋白質有951個胺基酸。帶有4個DM14的串聯重複，及1個C2結構域
- 功用：調節生物信號傳遞、免疫反應及神經發育
- 未有醫學文獻記錄它與左右軸排列和纖毛功能的關聯

***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
- Gene functions: regulation of signalling pathway, immune response & synapse maturation.
- Role in left-right axis formation and cilia function have never been reported

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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[1]

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[2]
- 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[3]

Mouse:
- *CC2D1A* knockout mouse is lethal, with cyanosis & breathing difficulties[1]

Human:
- Homozygous deletion involving exons 14 to 16 resulted in non-syndromic intellectual disability[2]
- Heterozygous deletion in 19p13.2-p13.12 (includes 6 other genes) resulted in developmental delay[3]

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

1. Oaks et al. Cereb Cortex. 2017 Feb 1;27(2):1670-1685
2. Basel-Vanagaite et al. J Med Genet. Mar 2006;43(3):203-10
3. Natiq et al. Mol Cytogenet. 2014;7:40

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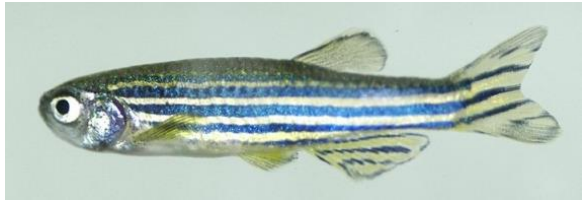
斑馬魚是心血管疾病研究所採用的優秀模型
Zebrafish as a good model for cardiovascular disease research

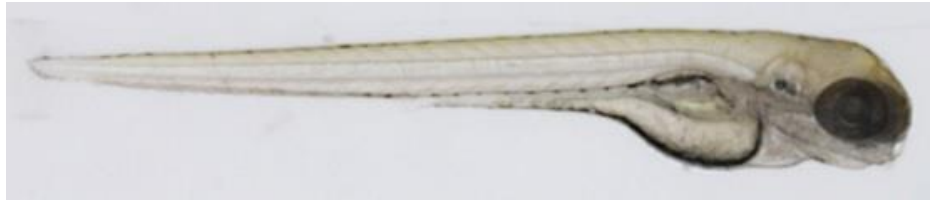
採用斑馬魚來進行心血管疾病研究的優點[1,2]：
- 胚胎時期是透明的
- 生育能力高，可以在實驗室內同時進行大量培殖 → 方便進行大規模篩選
- 斑馬魚和人類的心臟發展過程相似
- 由受精卵發育成為胚胎只需要24小時，當中包括心臟的發展

Advantages of using zebrafish for research[1,2]:
- Transparent during development
- High fertility & easy to house in a large quality ➔ 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

1. Poon KL et al. Glob Cardiol Sci Pract. 2013(1): 9–28
2. Nguyen et al. Drug Discov Today Dis Models. 2008 ; 5(3): 135–140



Zebrafish



Zebrafish embryo

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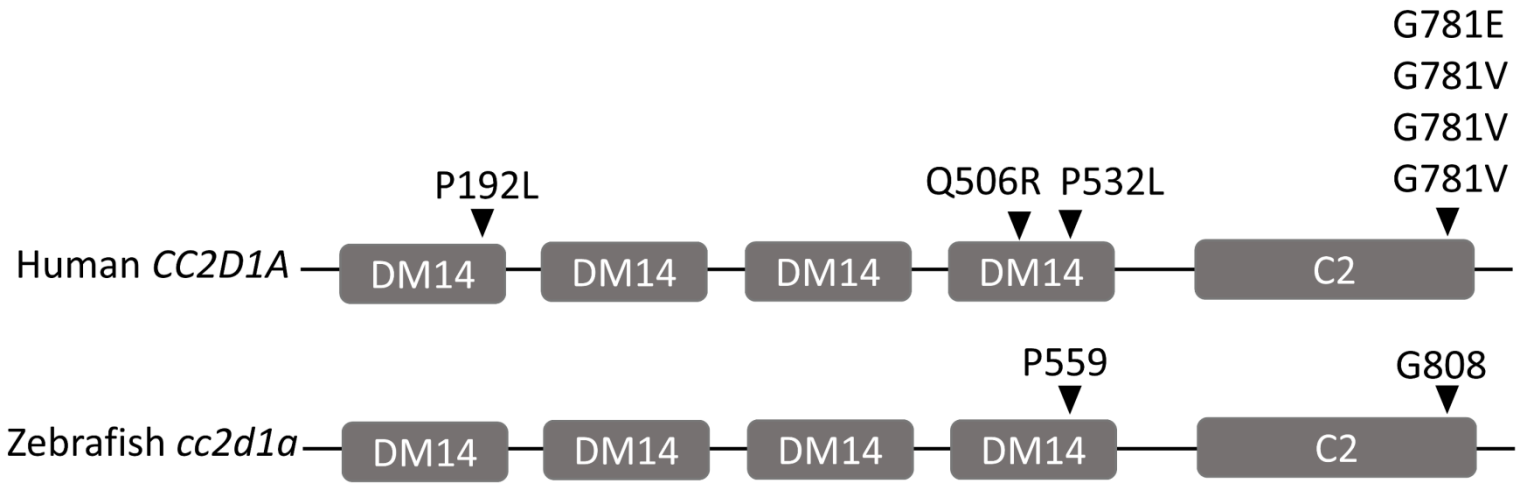
*CC2D1A*在人類和斑馬魚中是高度相似
CC2D1A is highly conserved in human and zebrafish



Human	**Zebrafish**
CC2D1A^{P532L}	= *cc2d1a*^{P559L}
CC2D1A^{G781V}	= *cc2d1a*^{G808V}

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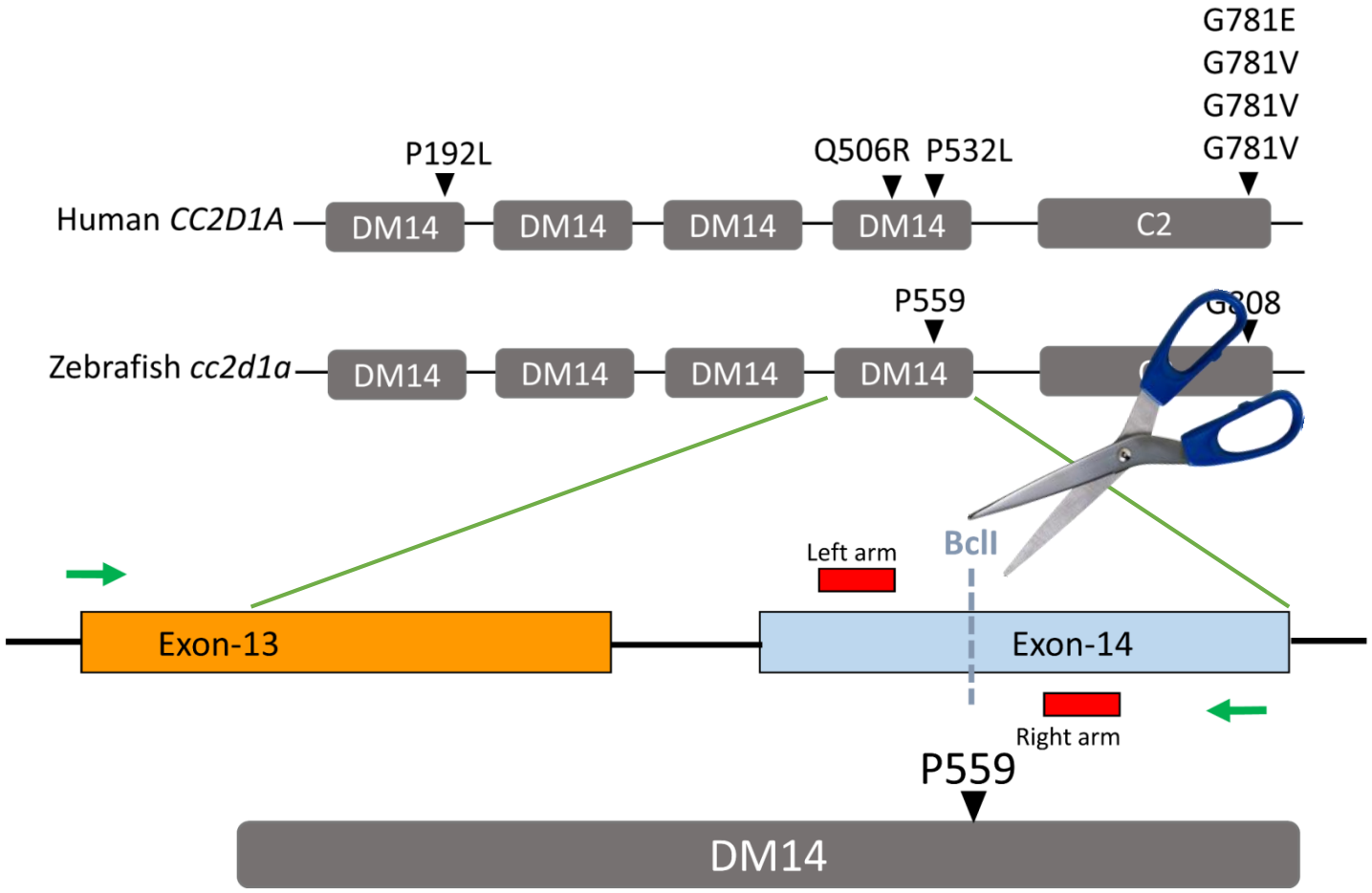
透過基因編輯技術刪除斑馬魚的*cc2d1a*
Creating a knock-out *cc2d1a* zebrafish model by genome editing



透過基因編輯技術刪除斑馬魚的*cc2d1a*，
令它的蛋白質失去正常功能

Cutting DNA of zebrafish *cc2d1a* by gene editing (TALEN), leading to an abnormal protein that lost its functions

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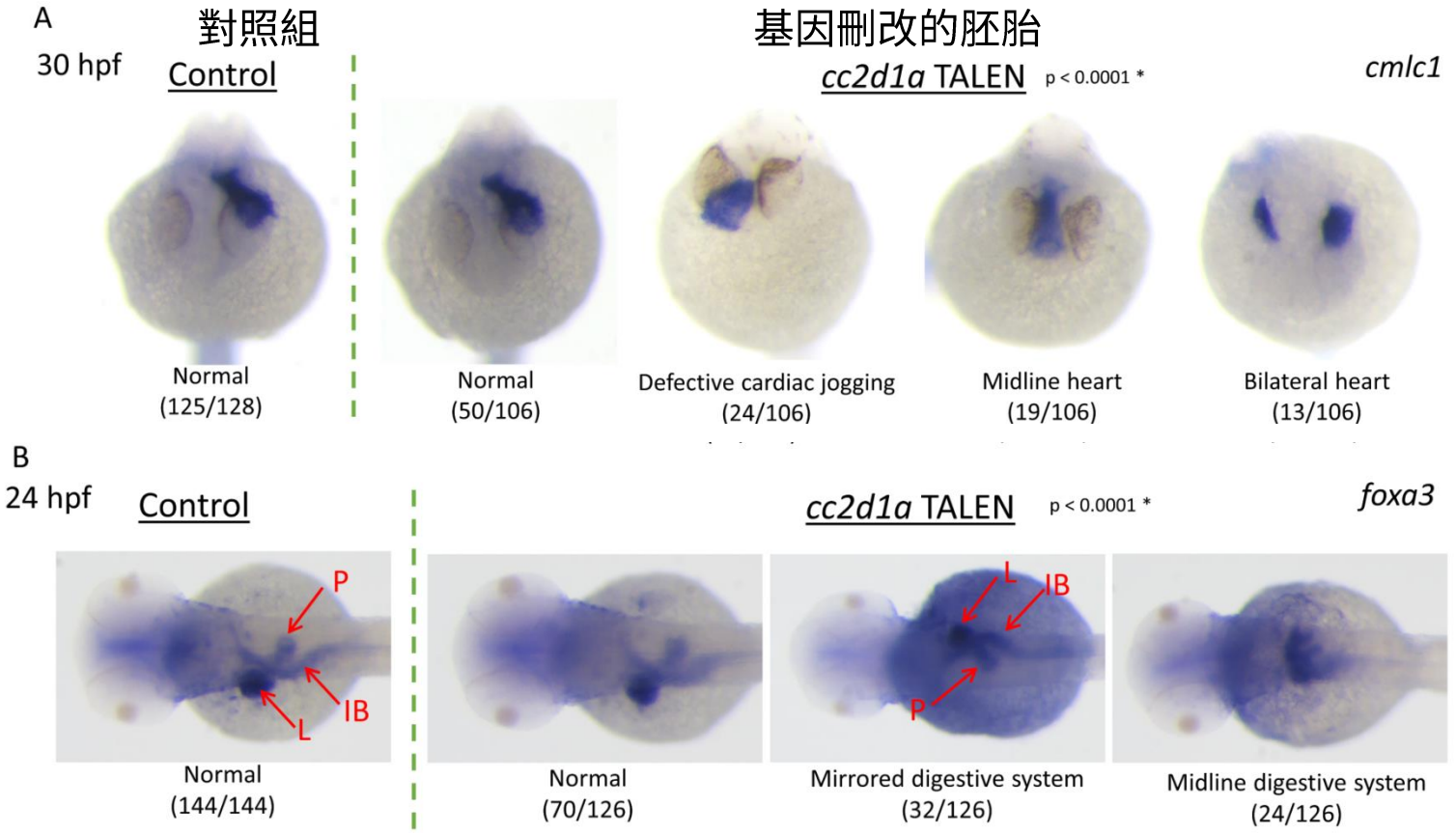
cc2d1a 的異變導致斑馬魚心臟和消化系統異常
Zebrafish with *cc2d1a* mutation showed heart & digestive system disarrangement



心臟
Heart

消化系統
Digestive system

注射沒有異變的*cc2d1a*到基因編輯的胚胎可以令器官排列回復正常

Injections of wild type *cc2d1a* to the edited fertilised egg can rescue the abnormal organ arrangement

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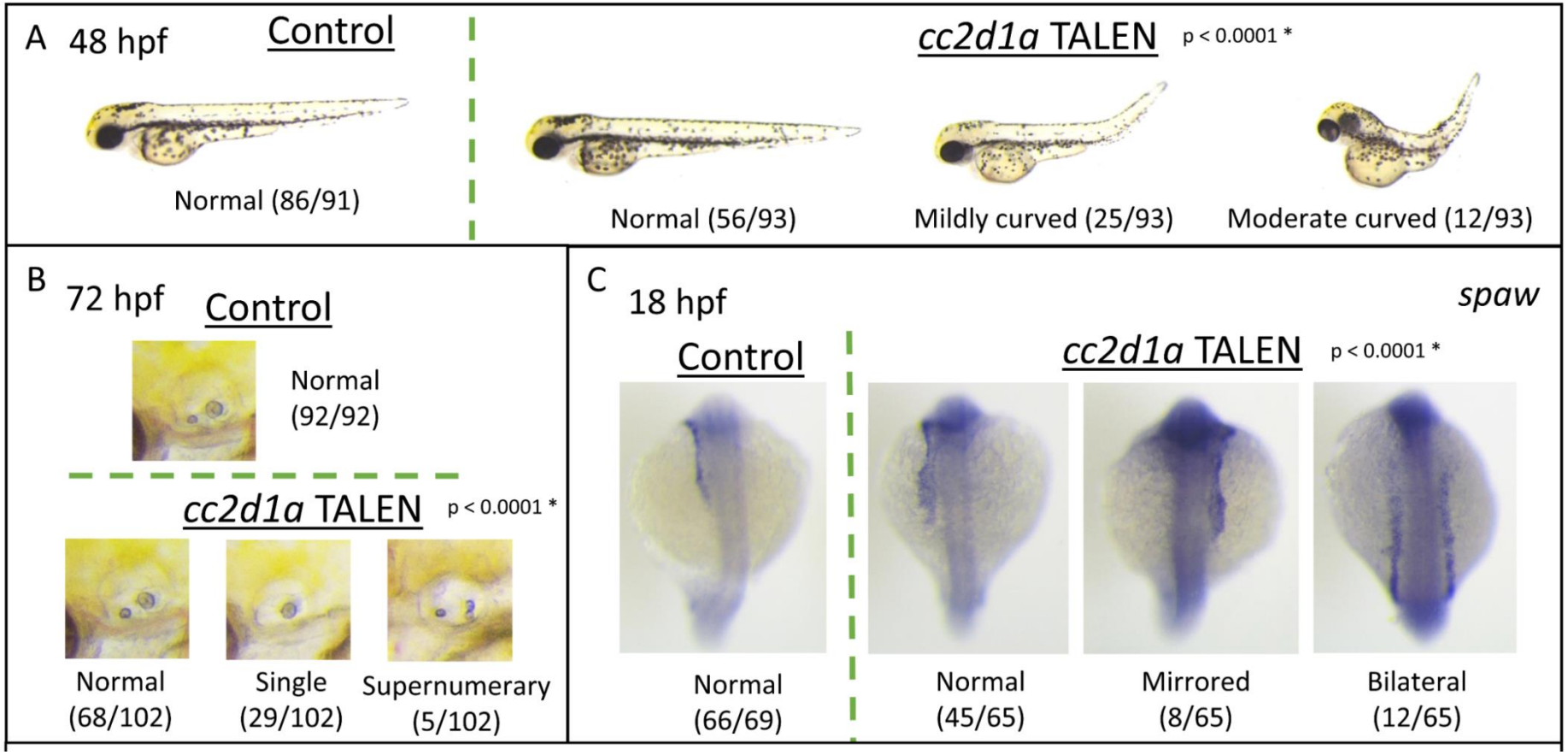
cc2d1a 的異變與纖毛缺陷相關
Mutations in *cc2d1a* were associated with cilia defects



腹側體軸
Ventral body axis

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

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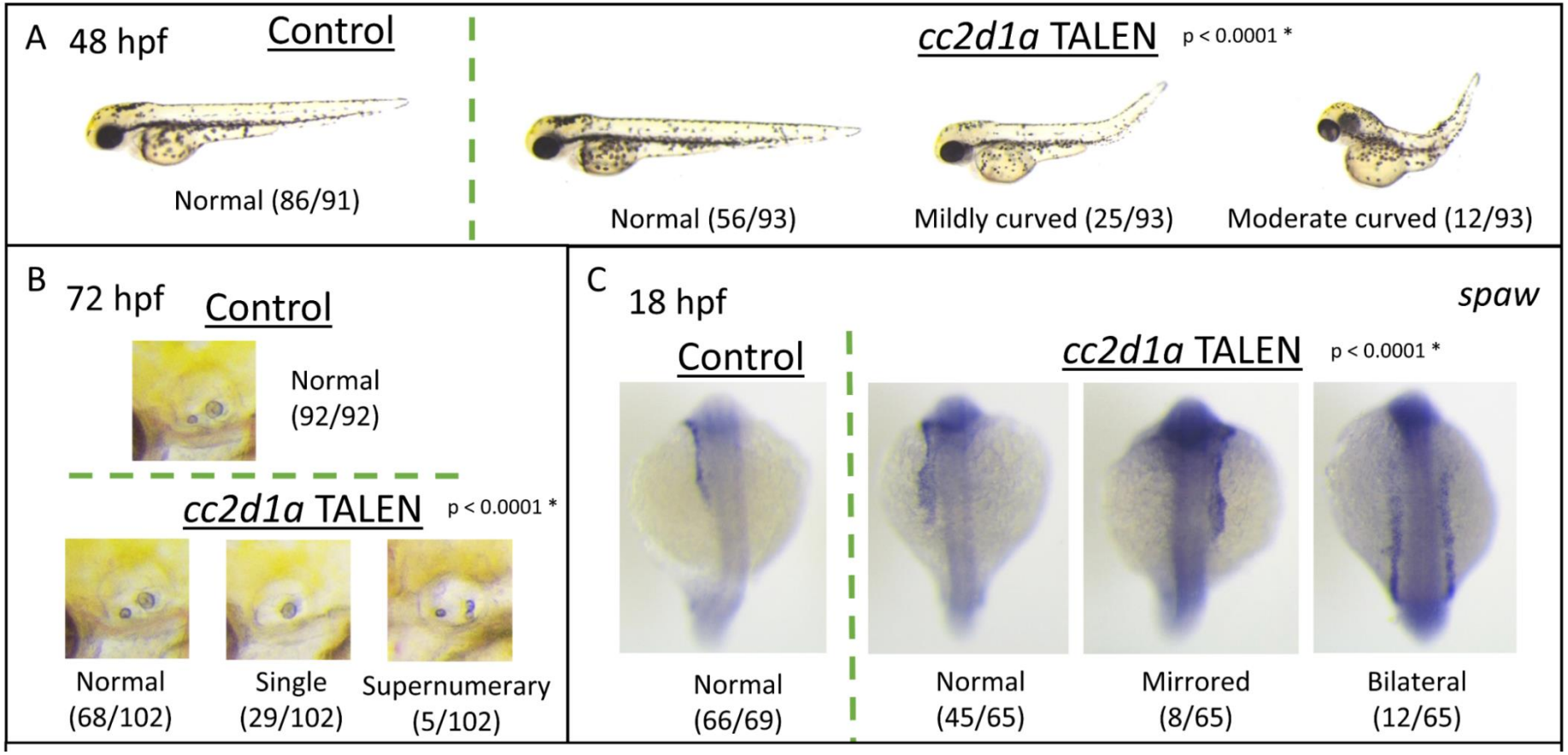

注射沒有異變的*cc2d1a*到基因編輯的胚胎可以令纖毛回復正常

Injections of wild type *cc2d1a* to the edited fertilized egg can rescue the cilia defects

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cc2d1a 的異變令斑馬魚細胞的纖毛減少及變短

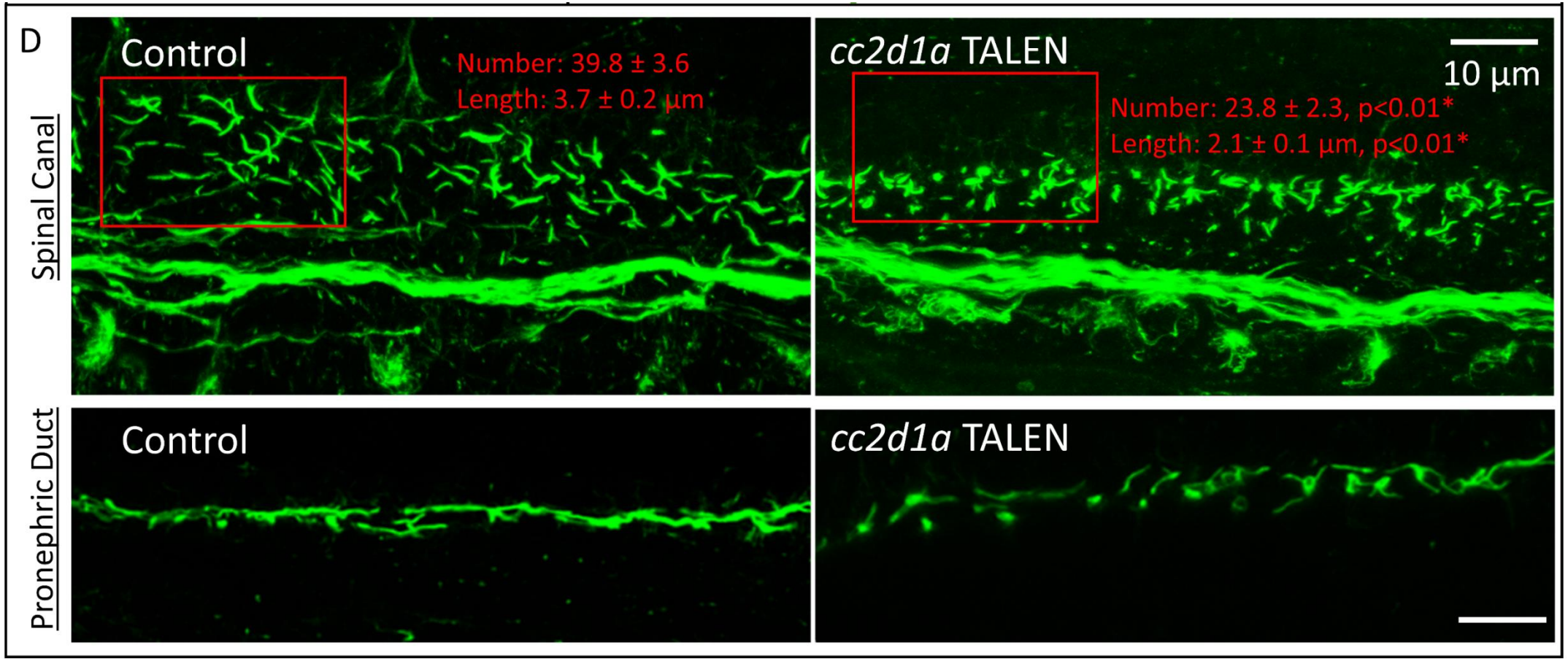
Mutations in *cc2d1a* were associated with fewer & shorter cilia in zebrafish cells

對照組

基因編輯的胚胎

椎管

原腎管



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總結
Summary

- *CC2D1A – 首次發現可導致異位綜合症*
  - 在26位異位綜合症的病人中，發現6位帶有相關的基因異變（19%）
  - 與非症候群型的先天性心臟病有關聯
  - 形成異位綜合症的原理與纖毛缺陷有關

- *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

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應用潛能 及 未來方向
Translational potentials & future directions

- 可以為有家族病史的病人進行有關CC2D1A的胚胎植入前基因診斷
- 可以進行產前胎兒診斷，但帶有相關的基因異變不一定會構成疾病，及疾病的嚴重程度亦仍難以估計
- 需要為帶有*CC2D1A*異變的病人檢查身體各個器官(特別是與呼吸系統有關的器官)有否受到相關的纖毛缺陷影響
- 美國心臟協會在2018年更新了有關先天性心臟病的科學準則，當中特別提及手術後的呼吸系統併發症對手術的成功率有關鍵的影響。如果帶有*CC2D1A*異變的病人有呼吸系統纖毛缺陷，醫生便可以針對他們呼吸系統的問題來提供度身訂造的治療，從而提升手術成功率[1]

- 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 possible[1]

American Heart Association®

AHA SCIENTIFIC STATEMENT

Genetic Basis for Congenital Heart Disease: Revisited

A Scientific Statement From the American Heart Association

Endorsed by the American Academy of Pediatrics

1. Pierpont ME et al. Circulation. 2018 Nov 20;138(21):e653-e711.

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