

# Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is “None”, please specify “None” as your response. “Not applicable” is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** University of Pittsburgh- of the Commonwealth System of Higher Education
2. **Reporting Period (start and end date of grant award period):** 1/1/2011-12/31/2014
3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Margaret C. McDonald, PhD
4. **Grant Contact Person’s Telephone Number:** 412-383-7474
5. **Grant SAP Number:** 4100054875
6. **Project Number and Title of Research Project:** 2 - Complex Genetics of Congenital Heart Disease
7. **Start and End Date of Research Project:** 1/1/2011-12/31/2014
8. **Name of Principal Investigator for the Research Project:** Cecilia W. Lo, PhD
9. **Research Project Expenses.**

9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

\$ 2,177,464.00

9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Lo, Cecilia	Professor	10% 2011, 10% 2012, 10% 2013, 10% 2014	\$82,801.20
Srinivasan, Ashok	Research Asst Professor	50% through 5/12; then 0%	\$64,125.00
Cooper, Jason	Research Technician	90% June – December 2011; then 0%	\$26,153.09
Li, You	Postdoctoral fellow	100% Jan – June, 2011; 0% 2012 and 2013; 100% 2014	\$65,508.78
Khalifa, Omar	Research Coordinator	100% in 2012, 2013, 2014	\$90,911.22
Damerla, Rama	Postdoctoral fellow	100% in 2011, 50% in 2013, 100% in December 2014	\$119,328.23
Zahid, Maliha	Research Associate	100% 2011, 100% 2012, 100% 2013, 100% 2014	\$37,335.38

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Stereomicroscope	For analysis of zebrafish embryos and respiratory airway cilia	\$5,614.30
Nanodrop	For estimating human patient DNA concentration	\$8,881.04
Ion Torrent	For amplicon resequencing for mutation recovery from patient DNA	\$5,859.45
Extracellular recording system	For analysis of nitric oxide from respiratory airway epithelia	\$9,500
NEXUS Mastercycler	For polymerase chain reaction (PCR) amplification of human DNA for Sanger sequencing validation of mutations	\$13,290.93

Nucleofector 2B	For transfection of small interfering RNA (siRNA) in respiratory epithelia for gene function assays	\$12,620.00
Cryopius and racks	For storage of human DNA and cells obtained from the congenital heart defect (CHD) patients	\$10,052.61

**10. Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes \_\_\_\_\_ X \_\_\_\_\_ No \_\_\_\_\_

If yes, please indicate the source and amount of other funds:

1. University of Pittsburgh School of Medicine Start-Up Funds: \$300,000
2. NIH R01 GM104412

Grant Title: Assaying Heterotaxy Patient Genes in Cilia Motility and Left-Right Patterning  
Amount: \$380,119

**11. Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes \_\_\_\_\_ X \_\_\_\_\_ No \_\_\_\_\_

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert “not funded” in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
Assaying Heterotaxy Patient Genes in Cilia Motility and Left-Right Patterning	<input checked="" type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify:_____) <input type="checkbox"/> Nonfederal source (specify:_)	March 2013	\$ 500,000	\$383,000
Respiratory Ciliary Dysfunction and Pulmonary Risks in Congenital Heart Disease	<input type="checkbox"/> NIH <input checked="" type="checkbox"/> Other federal (specify: Department of Defense) <input type="checkbox"/> Nonfederal source	October 2013	\$351,003	\$ under review
Airway Ciliary Dysfunction and Worse Respiratory and Neurodevelopmental Outcomes in Congenital Heart Disease Patients	<input type="checkbox"/> NIH <input checked="" type="checkbox"/> Other federal (specify: Department of Defense) <input type="checkbox"/> Nonfederal source (specify:_)	November 2014	\$1,294,975	\$ under review
Ciliary Dysfunction and Poor Neurodevelopmental Outcome in Congenital Heart Disease	<input checked="" type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify:_____) <input type="checkbox"/> Nonfederal source (specify:_)	February 2013	\$505,192	\$ under review

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes  No

If yes, please describe your plans:

We plan to submit a program project grant to NIH in May 2015 that will focus on the pathogenesis of hypoplastic left heart syndrome. This project will build on some of the exome sequencing data generated from this study.

**12. Future of Research Project.** What are the future plans for this research project?

We are seeking funding with grant applications submitted to the U.S. Department of Defense and the National institutes of Health.

**13. New Investigator Training and Development.** Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes  No

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				2
Female				
Unknown				
<b>Total</b>				<b>2</b>

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				2
Unknown				
<b>Total</b>				<b>2</b>

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				2
Other				
Unknown				
<b>Total</b>				<b>2</b>

**14. Recruitment of Out-of-State Researchers.** Did you bring researchers into Pennsylvania to carry out this research project?

Yes  No

If yes, please list the name and degree of each researcher and his/her previous affiliation:

Jason Cooper, BS - American Type Culture Collection (ATCC) - Manassas, Virginia

**15. Impact on Research Capacity and Quality.** Did the health research project enhance the quality and/or capacity of research at your institution?

Yes  No

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

Our study has developed in-house expertise for computation analysis of next-generation human exome sequencing data.

## 16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes \_\_\_\_\_ No  X

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes \_\_\_\_\_ No  X

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes \_\_\_\_\_ No  X

If yes, please describe involvement with community groups that resulted from the research project:

## 17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

**There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha ( $\alpha$ ) and beta ( $\beta$ ) should not print as boxes ( $\square$ ) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.**

The goal of our study is to interrogate the potential contribution of mutations in cilia-related genes in the pathogenesis of congenital heart defects (CHD) and in the unexplained postsurgical respiratory complications often seen in CHD patients. We began with a focus on CHD associated with heterotaxy, a birth defect characterized by discordant abdominal and thoracic organ situs because of aberrant embryonic left-right patterning. In fact, the heart is the most left-right asymmetric organ in the body. Thus, it is not surprising that some of the most complex CHD are observed in heterotaxy patients. This heart asymmetry is essential for establishing pulmonary versus systemic circulation required for efficient blood oxygenation. Because this developmental process is dependent on motile cilia situated in the embryonic node (and motile cilia are also required for mucociliary clearance in the airway), our study examined the hypothesis that CHD patients may suffer from mutations that disrupt motile cilia function required for both embryonic left-right patterning and airway clearance. This condition could explain the high prevalence of postsurgical respiratory complications seen in CHD patients. The observation that mice and patients with mutations causing respiratory ciliary dysfunction can exhibit both sinopulmonary disease (known as primary ciliary dyskinesia [PCD]) and left-right patterning defects, including heterotaxy accompanied by CHD, is consistent with our hypothesis.

Therefore, our goal in this project was to assess the potential contribution of cilia-related mutations in airway ciliary dysfunction and increased pulmonary morbidity in CHD patients. We expanded our analysis and have uncovered a similar high prevalence of airway ciliary dysfunction in CHD patients without heterotaxy. Using next-generation sequencing, we obtained whole-exome sequencing data for 250 CHD patients. For an additional 160 CHD patients with heterotaxy, we obtained targeted resequencing data using the Ion Torrent next-generation sequencing platform. This analysis showed that CHD patients with ciliary dysfunction are enriched for mutations in cilia-related and PCD genes, suffer more respiratory symptoms and disease, and experience more postsurgical pulmonary morbidities. We have also conducted functional validation experiments using tissue culture studies and animal model experiments to validate the candidate cilia-related genes identified in our CHD patient sequencing analysis. These findings are provided below in more detail. Together, these observations complete the goals outlined in our three aims.

**Aim 1.** We will identify possible disease causing sequence variants using whole-exome capture and next-generation sequencing in 50 patients with complex congenital heart disease and heterotaxy.

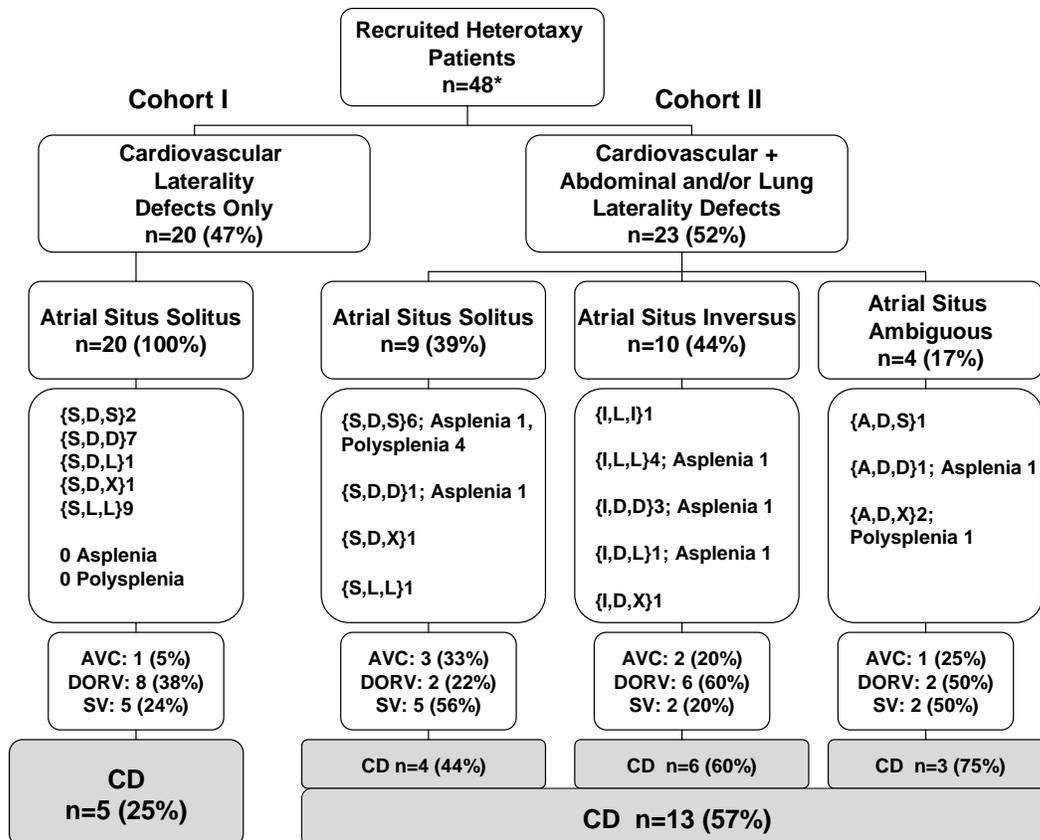
**Aim 2.** Sequence variants recovered in the ciliome and congenital heart disease genes will be interrogated using online repositories such as dbSNP or HapMap to identify those that are rare variants or mutations, and these will be validated by capillary sequencing of the patient DNA and further interrogated against sequencing data from the 1,000 Human Genome Project and other publicly available human exome/whole genome data sets.

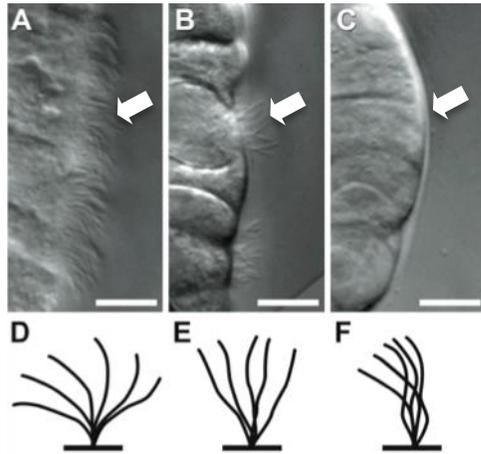
**Aim 3.** To validate rare sequence variants recovered in the CHD patients as disease causing, we will conduct exome capture and SOLiD sequencing of a replication cohort of 200 patients with CHD to be recruited from Children's Hospital of Pittsburgh of UPMC.

Publication: **High prevalence of respiratory ciliary dysfunction in heterotaxy patients with congenital heart disease (Nakhleh et al, 2012).** *Circulation.* 2012; 125: 2232-2242 Published online before print April 12, 2012, doi: 10.1161/CIRCULATIONAHA.111.079780

We recruited 48 CHD patients with heterotaxy (see Figure 1), 42 of whom completed the study. We recorded CHD phenotype and medical history for each participant. Nasal scrapes were obtained, and high-speed videomicroscopy was used to assess respiratory ciliary motion (Figure 2). In participants with normal ciliary motion, we observed synchronous, metachronal waves of coordinated ciliary beat that were identical to those seen in healthy controls (Figure 2). However, in PCD patients and in some of the CHD/heterotaxy patients, we observed dyskinetic ciliary motion characterized by stiff, wavy, or incomplete strokes. In some cases, cilia were completely immotile (Figure 2). We also observed an absence of cilia in some patients (Figure 2C). From this analysis, we identified 18 of 42 (42 percent) participants with abnormal ciliary motion, referred to as patients with ciliary dysfunction (CD) (Figure 1). Those patients > 6 years of age with CD were assessed for respiratory symptoms and found to have had more respiratory symptoms and disease (Table 1), including recurrent otitis media, neonatal respiratory distress, and bronchiectasis—presentations typically associated with PCD. Among the CHD patients who underwent cardiac operations, we found those with CD suffered more postsurgical respiratory complications (Table 2), consistent with the notion that respiratory CD may compromise mucociliary clearance function in CHD patients and pose increased risk for postsurgical pulmonary morbidity.

**Figure 1. Laterality and Cardiovascular Defects in Heterotaxy Patients**





**Figure 2. Ciliary motion in nasal epithelia from heterotaxy and PCD patients**

(A). Abundant cilia (arrow) were observed in patient 9033 exhibiting normal ciliary motion.  
 (B). Paucity of cilia (arrow) in CHD patient 9027 with CD.  
 (C). No cilia found in nasal epithelia (arrow) of patient 9004.  
 (D). Healthy control showing normal motion with full forward and recovery strokes.  
 (E). PCD patient 9028 has stiff motion with shortened stroke and minimal ciliary bending.  
 (F). CHD patient 9011 with heterotaxy and CD exhibits shortened forward stroke and wavy recovery stroke with limited motion.

**Table 1. Respiratory manifestations in heterotaxy patients\***

	Age	Gender	Recurrent otitis media	Recurrent lower respiratory illnesses	Neonatal respiratory distress	Chronic wet cough	Chronic nasal congestion	Chronic sinusitis	Respiratory insufficiency/tracheotomy	Bronchiectasis
<b>&gt; 6 years perioperative</b>										
CD-A	9011	12 yr	F	N	N	N	N	N	N	N
	9013	7 yr	F	N	Y	N	N	N	N	N
	9016	19 yr	F	N	N	N	N	N	N	N
	9046	9 yr	F	Y	N	N	N	N	N	N
<b>nonperioperative<sup>†</sup></b>										
CD-A	9003	26 yr	F	Y	Y	Y	N	Y	N	Y
CD-A	9020	46 yr	F	Y	Y	N	Y	N	N	N
CD-A	9032	19 yr	M	N	N	N	N	N	N	N
CD-A	9037	18 yr	M	Y	N	Y	Y	Y	N	N
CD-B	9008	10 yr	M	N	N	N	N	N	N	N
CD-B	9026	18 yr	F	N	N	N	N	N	N	N
CD-B	9027	6.5yr	F	N	Y	Y	Y	Y	N	N
CD-B	9031	15 yr	M	Y	Y	N	N	Y	Y	N
	9009	30 yr	F	N	N	N	N	N	N	N
	9019	44 yr	M	N	Y	N	Y	N	Y	N
	9033	29 yr	M	N	N	N	N	N	N	N
	9035	28 yr	F	N	N	N	N	N	N	N
	9036	25 yr	M	N	N	N	N	Y	Y	N
	9038	50 yr	F	N	Y	N	N	Y	N	N
	9042	12 yr	F	N	N	N	Y	Y	N	N

Pink versus yellow highlighting denotes respiratory symptoms in CD versus no-CD patients, respectively.

<sup>†</sup>Wilcoxon rank sum test shows P=0.024 for number of respiratory manifestations in nonperioperative CD versus no-CD patients.

**Table 2. Respiratory Outcome Measures by Surgical Encounter (from Harden et al., 2013).**

	CD (n=25)	No-CD (n=27)	p value
	Median (IQR <sup>A</sup> )	Median (IQR)	
Mechanical Ventilation (days)	1 (1.0 – 3)	1 (1 – 3)	0.69
Chest Tube Length (days)	3 (2 – 6)	3 (2 – 7)	0.98
	No. (%)	No. (%)	
Prolonged Ventilatory Course (≥ 10 days)	5 (20)	2 (7)	0.24
Respiratory Complications	19 (76)	10 (37)	<b>0.006</b>
Total Tracheostomies <sup>#</sup>	4 (16)	0 (0)	<b>0.047</b>
New Tracheostomies	2 (9)	0 (0)	0.21
Inhaled β-agonists use	16 (64)	3 (11)	<b>0.001</b>

A: IQR = interquartile range; <sup>#</sup> Includes new tracheostomies and existing tracheostomies required for ventilation

Blood was obtained for each patient, and DNA was extracted for sequencing analysis. We conducted whole-exome sequencing using Agilent SureSelect All Exon Kit and Illumina HiSeq2000 with 100bp pair-end sequencing to achieve 100x target sequence coverage. Sequence reads were aligned to the reference human genome using Burrows-Wheeler aligner followed by GATK version 3 Best Practice (<https://www.broadinstitute.org/gatk/guide/best-practices>) for post-processing and variant calling. All sequence variants were annotated with mutation-effect prediction (SNPEff, Annovar), allele frequencies (1,000 Genomes, NHLBI EVS), and significance prediction scores (SIFT, Polyphen2, and CADD), using the CADD C-score to set or adjust the stringency of variant selection. Using the processed exome sequencing data, we conducted a burden analysis focused on two candidate gene lists—genes known to cause PCD and a ciliome gene derived from the Syscilia database (<http://www.syscilia.org/goldstandard.shtml>).

Sequencing analysis was carried out for 13 heterotaxy patients with CD, 12 heterotaxy patients without CD, 10 PCD disease controls, and 13 healthy controls. Four PCD patients were found to have two known PCD causing mutations. Sequencing analysis of the 13 heterotaxy patients with CD revealed one individual (9002) heterozygous for the *DNAI1* founder mutation, IVS1+2\_3insT, known to cause PCD. Another participant (9026) had three novel mutations, two in *DNAH5* and one in *DNHA11*. Participant 9003 exhibited two *DNAH11* mutations. This pattern was associated with hyperkinetic ciliary beat, a phenotype unique to PCD arising from *DNAH11* mutations. Overall, the highest incidence of novel coding variants (NCVs) was observed in the PCD patients—one NCV/participant (Figure 4), followed by heterotaxy patients with CD yielding 0.769 NCV/participant, then heterotaxy patients with no-CD with 0.417 NCV/participant (Figure 4). In contrast, the healthy controls had a single variant, yielding 0.077 NCV/participant. Statistical analysis of the percentage of patients with NCV and the mean number of NCV per patient showed significant differences for heterotaxy patients with CD and the PCD patients when compared to controls (Figure 4). This observation is not accounted for by differences in racial composition, as Fisher's exact test for race-ethnicity between the controls versus heterotaxy patients with CD yielded  $p=0.33$  (Table 4).

We noted four of the 10 PCD patients did not have mutations in any of the 14 PCD genes, suggesting additional novel PCD genes are yet to be identified. One heterotaxy patient with CD had a *DNAI1* founder mutation known to cause PCD, while four PCD patients (three unrelated) had two known PCD causing *CCDC39* or *CCDC40* mutations. Since PCD is a recessive disorder, PCD-causing mutations are expected to be homozygous or compound heterozygous. Consistent with this expectation, three PCD patients were homozygous or compound heterozygous for PCD-causing *CCDC39* or *CCDC40* mutations. In contrast, no heterotaxy patients had two known PCD mutations. For five heterotaxy patients with CD and two PCD patients, only a single heterozygous PCD gene mutation was observed. This group included PCD patient 564 (with a known disease causing *CCDC40* mutation) and heterotaxy patient 9002 with the *DNAI1* founder mutation. In heterotaxy patient 9026, a *DNAH5/DNAH11* double heterozygous mutation was observed. We hypothesize that PCD or CD in heterotaxy patients may arise from two mutations—one in each of two different cilia-related genes. This outcome may involve the combined effects of mutations in PCD genes and other cilia related genes not associated with PCD. Such multigenic etiology and genetic heterogeneity may underlie the phenotypic differences in the ciliary dysfunction seen in heterotaxy versus PCD patients.

Overall, these findings suggest CHD patients with heterotaxy have substantial risk for CD and respiratory disease. This outcome may involve mutations in novel and known PCD genes. These findings suggest CHD patients with heterotaxy may benefit from preoperative screening for CD. Further studies are needed to evaluate whether therapies enhancing mucus clearance may reduce respiratory complications and improve postsurgical outcomes for CHD patients with CD.

**Table 3. Novel Variants in PCD Genes in CHD Patients with Heterotaxy**

Patient	Ethnicity	Function*	Gene	Base Change <sup>†</sup>	Amino Acid <sup>‡</sup>
9002	White	CD	<i>DNAI1</i>	IVS1+2_3insT <sup>†</sup>	Truncation
9003	African American	CD	<i>DNAH11</i>	4520A>C	Q1507P <sup>‡</sup>
			<i>DNAH11</i>	9397G>A	E3133K <sup>‡</sup>
9004	Asian	CD	<i>TXNDC3</i>	1630G>A	A544T <sup>‡</sup>
9006	White	CD	<i>CCDC39</i>	626C>G	A209G <sup>‡</sup>
9008	African American	CD	None		
9011	White	CD	None		
9015	White	CD	<i>LRRC50</i>	1294G>A	E432K
9017	African American	CD	None		
9018	African American	CD	None		
9026	African American	CD	<i>DNAH11</i>	9203A>G	E3068G <sup>‡</sup>
			<i>DNAH5</i>	11140A>G	I3714V
			<i>DNAH5</i>	638C>A	P213Q <sup>‡</sup>
9027	White	CD	None		
9031	White	CD	None		
9037	African American	CD	<i>DNAI1</i>	1579T>G	S527A <sup>‡</sup>
9005	African American	no-CD	None		
9007	African American	no-CD	None		
9009	White	no-CD	None		
9012	African American	no-CD	None		
9016	White	no-CD	None		
9019	African American	no-CD	<i>DNAH5</i>	6710A>G	N2237S
9024	African American	no-CD	None		
9025	Asian	no-CD	<i>DNAI1</i>	1795G>A	A599T <sup>‡</sup>
			<i>DNAI1</i>	2054T>C	L685P <sup>‡</sup>
9033	White	no-CD	<i>CCDC39</i>	1865A>G	E622G
			<i>DNAI1</i>	1177G>A	V393M
9035	White	no-CD	None		
9040	African American	no-CD	None		
9068	White	no-CD	None		

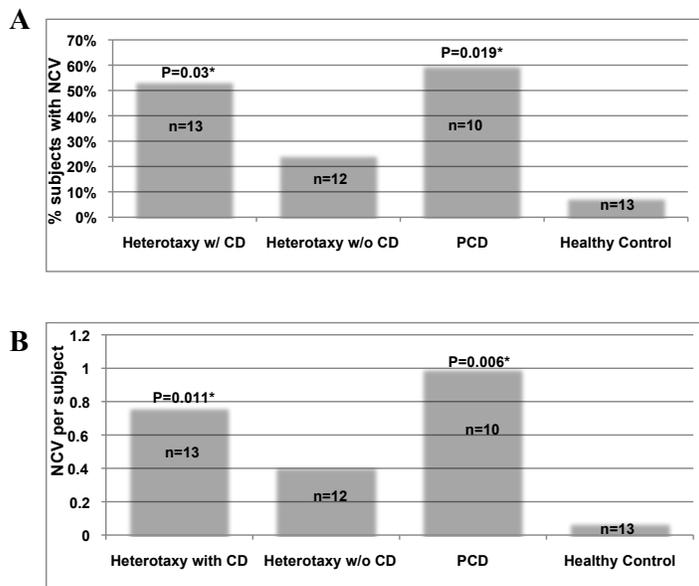
CD: ciliary dysfunction; PCD: primary ciliary dyskinesia. Het: heterozygous mutation; homo: homozygous mutation

**Table 4. Novel Coding Variants in Heterotaxy and PCD Patients\***

Patient Type	Total Subjects	With NCVs (%) <sup>†</sup>	Number NCVs	NCVs Per Subject <sup>‡</sup>
Heterotaxy with CD	13	7 (54%) p=0.019	10	0.769 p=0.011
Heterotaxy without CD	12	3 (25%)	5	0.417
PCD	10	6 (60%) p=0.03	10	1.000 p=0.006
Healthy Controls	13	1 (8%)	1	0.077

\*p-value <0.025 was considered statistically significant for each comparison based on the Bonferroni correction. The Fisher's exact test and the Kruskal Wallis test were used for comparisons of the presence and the number of NCVs in the four groups; both tests yielded significant results ( $P=0.021$  and  $P=0.024$ , respectively).

**Figure 4. Novel Coding Variants in Heterotaxy and PCD Patients**



Publication: **Airway ciliary dysfunction and sinopulmonary symptoms in patients with congenital heart disease of a broad spectrum** (Garrod et al, 2014). Andrea S. Garrod, Maliha Zahid, Xin Tian, Richard J. Francis, Omar Khalifa, William Devine, George C. Gabriel, Linda Leatherbury, and Cecilia W. Lo *Annals of the American Thoracic Society*, Vol. 11, No. 9 (2014), pp. 1426-1432. doi: [10.1513/AnnalsATS.201405-222OC](https://doi.org/10.1513/AnnalsATS.201405-222OC)

CHD patients with heterotaxy exhibit high prevalence of airway CD, a reflection of the role of motile cilia in airway clearance and left-right patterning. However, we have uncovered a broader role for ciliome mutations in the pathogenesis of CHD in a large-scale mouse mutagenesis screen. In our screen, 46 of 90 CHD-causing genes we recovered were identified as ciliome genes, indicating ciliome mutations may have a much larger role in CHD pathogenesis. Hence, we undertook the assessment of a broad spectrum of CHD patients without heterotaxy to determine the potential clinical significance of airway CD.

Patients with a broad spectrum of CHD were recruited (n=218; Figure 3), 180 without heterotaxy. We obtained medical history and nasal scrapes for ciliary motion videomicroscopy to assess respiratory motile cilia function. We also obtained patient history of sinopulmonary symptoms and disease. This analysis revealed a high prevalence of ciliary motion defects (51.8 percent) in CHD patients with or without heterotaxy. Patients with CD showed increased sinopulmonary symptoms (Table 5). Multivariate analysis showed that CD was more important in determining risk of sinopulmonary symptoms than heterotaxy status. Sinopulmonary disease seen in CHD patients (such as chronic cough or neonatal respiratory distress) has frequently been attributed to underlying cardiac defects or comorbid risk factors. Thus, these patients are usually not further considered for assessment of respiratory cilia defects.

Based on the findings of this study, we propose that symptomatic CHD patients should be referred to a pulmonologist for further assessment of mucociliary clearance impairment. Future studies are needed to explore possible therapies to reduce respiratory morbidity in this fragile patient group. With specific medical interventions, sinopulmonary complications in the post-operative period may be reduced. Morbidity might also be reduced during the patient's lifetime in the growing population of adults with CHD.

Covariates	All symptoms		PCD symptoms		Upper symptoms		Lower symptoms	
	exp(b)	<i>P-value</i>	exp(b)	<i>P-value</i>	exp(b)	<i>P-value</i>	exp(b)	<i>P-value</i>
Age	0.996	0.44	0.99	0.2	1.006	0.42	0.982	<b>0.048</b>
HTX vs. non-HTX	1.103	0.51	1.067	0.75	1.223	0.31	0.973	0.90
CD vs. No CD	1.426	<b>0.006</b>	1.423	<b>0.043</b>	1.178	0.4	1.786	<b>0.003</b>

**Table 5. Multivariate Analysis of CD and Respiratory Symptoms\***

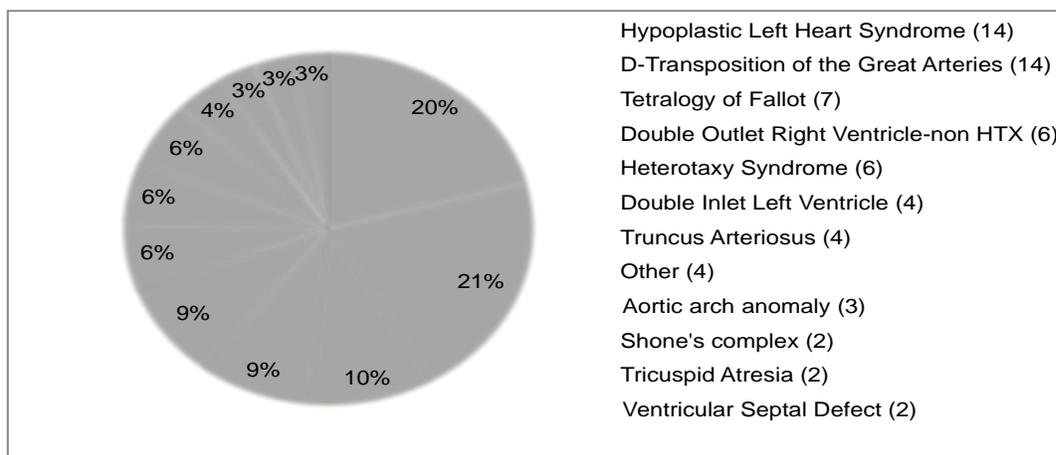
\*In each log-linear regression model, the regression coefficient exp ( $\beta$ ) represents one unit increase in the covariate x as a multiplicative effect on the mean of the outcome. When exp ( $\beta$ ) >1, the mean of the outcome increases as x increases; if exp ( $\beta$ ) <1, the mean of the outcome decreases as x increases. Significant P values are bolded.

Submitted for publication: **Airway Ciliary Dysfunction and Postoperative Complications in Congenital Heart Disease Patients (Stewart et al, 2015)**

We showed that CHD patients with or without heterotaxy have a high prevalence of respiratory CD made up of abnormal airway ciliary motion similar to that seen with PCD. Heterotaxy patients with CD have more postoperative morbidity, including more respiratory complications; but the effects of CD on postoperative outcome in nonheterotaxy CHD patients are unknown. We hypothesize that non-heterotaxy CHD patients with CD have increased postoperative morbidity, particularly respiratory complications. To investigate this hypothesis, we recruited 69 patients younger than 1 year of age with a broad spectrum of CHD undergoing 106 cardiac operations. We conducted nasal nitric oxide (nNO) measurement, typically low with PCD, and obtained nasal epithelia for ciliary motion (CM) videomicroscopy. Data on post-operative outcome parameters were collected (Table 6). CHD patients exhibited a high prevalence of abnormal CM (32 percent) and low nNO (42 percent). Demographics and surgical-complexity scores were similar between groups. Patients with abnormal CM had more respiratory complications, including more days on positive-pressure ventilation (P=0.003), and more  $\beta$ -agonist (P=0.005) and Dornase-alfa (P=0.020) use. In contrast, patients with low nNO showed decreased systolic function (P=0.001), increased intensive-care unit stay (P=0.005), longer mechanical ventilation (P=0.028), increased intravenous inotropes and vasodilator use (P=0.004; P=0.027), and more infections (P=0.001).

Overall, these findings demonstrate that CHD patients with CD have significantly higher postoperative morbidity, with abnormal CM associated with poor respiratory function, while low nNO correlated with hemodynamics alterations. We observed that CHD patients with CD have an odds ratio (OR) of 10 for respiratory viral infection, 4.2 for positive-pressure ventilation, and 3.75 for inhaled  $\beta$ -agonist use. We note that while patients with PCD often exhibit abnormal CM and low nNO, only a small subset of our patients showed both low nNO and abnormal CM. This observation indicates that most CHD patients with CD do not have classic PCD. Our findings suggest that CD in CHD patients may drive changes in clinical outcomes through primary and secondary mechanisms acting in combination to affect motile and primary cilia function. Future prospective studies using preoperative evaluation for CD in CHD patients may identify at-risk patients, thereby allowing perioperative intervention to reduce morbidity following cardiac surgery. This method can help improve postsurgical outcome for this particular patient cohort with critical CHD, who frequently must undergo multiple high-risk cardiac operations to palliate their structural heart defects.

**Figure 3. Patients with a Broad Spectrum of CHD Phenotypes**



**Table 6. Effect of abnormal CM on CHD patients' postoperative outcomes\***

	All Surgical Events (n=106)		CHD Patients (n=102)	
			Normal CM (n=65)	Abnormal CM (n=37)
Postoperative LOS (days)	20.3±17.0	21.3 ±16.6	19.2 ±18.4	P = 0.173
Total CICU LOS (days)	9.2±7.1	9.6 ±7.1	8.8 ±7.4	P = 0.222
Initial CICU LOS (days)	8.5±6.4	8.9±6.3	8.2±6.7	0.346
ECMO (days)	0.3±0.8	0.3±0.9	0.3±0.8	P = 0.866
Delayed sternal closure (days)	1.7±1.8	2.0±1.9	1.3±1.6	P = 0.091
Mechanical ventilation (days)	4.4±3.1	4.6±3.2	4.2±3.0	P = 0.486
Supportive ventilation (days)	6.7±6.5	7.0±6.6	6.2±6.5	P = 0.279
Positive-pressure ventilation (days)	0.7±2.1	0.4±1.4	1.2±2.9	<b>P = 0.003</b>
Reintubation (n)	20 (19%)	10 (15%)	10 (27%)	P = 0.154
Albuterol (days)	0.9±3.1	0.4±1.3	1.8±4.9	<b>P = 0.005</b>
Dornase alfa (days)	0.2±1.0	0	0.4±1.5	<b>P = 0.020</b>
Ipratropium (days)	0.2±1.2	0	0.4±1.9	<b>P = 0.060</b>
Overall infection (n)	31 (29%)	17 (26%)	13 (35%)	P = 0.339
Bacterial infection (n)	26 (25%)	15 (23%)	11(30%)	P = 0.459
Respiratory viral infection (n)	6 (6%)	1 (2%)	5 (14%)	<b>P = 0.023<sup>†</sup></b>

\*P values obtained by Pearson  $\chi^2$  unless otherwise noted; <sup>†</sup>P value by Fisher's exact test

**Table 7. Risk of various postoperative outcomes associated with respiratory ciliary dysfunction**

	Odds Ratio	P - value	95% CI
<b>Ciliary Dysfunction</b>			
Positive-Pressure Ventilation	4.2	0.003	1.6-10.7
$\beta$ -Agonist Use	3.75	0.006	1.5-9.6
Respiratory Medication Use	3.5	0.006	1.4-9.3
Respiratory Viral Infection	10	0.039	1.1-89.2

CHD patients with heterotaxy, a birth defect involving randomized left-right patterning, have a high prevalence of CD and increased respiratory symptoms, likely reflecting the role of motile cilia in airway clearance and left-right patterning. We hypothesize that, like heterotaxy, transposition of great arteries (TGA) will have high prevalence of airway CD with more respiratory symptoms. TGA involves abnormal heart looping and has been suggested to arise from defects in left-right patterning. In dextro-TGA (D-TGA), ventriculo-arterial discordance occurs, with anterior aorta positioning and insertion into the right ventricle; while in levo-TGA (L-TGA), there is ventricular inversion, with the morphological right ventricle positioned on the body's left, also known as congenitally corrected TGA. Hence, TGA, especially L-TGA, is suggested to have a common developmental etiology with heterotaxy.

We recruited 75 CHD patients with isolated TGA, 28 percent L-TGA and the remainder D-TGA. Airway CD was assessed by measuring nNO, which is typically low with PCD. We also obtained nasal scrapes for videomicroscopy to assess for abnormal CM. Low nNO was observed in 29 percent and abnormal CM in 57 percent of TGA patients, with 22 percent exhibiting both low nNO and abnormal CM. No difference was observed between D versus L-TGA. Respiratory symptoms were increased with abnormal CM, but there was no correlation with either low nNO or TGA-type (Table 8). It is important to note that some respiratory symptoms, such as newborn respiratory distress, are likely underreported in CHD patients, as they may be attributed to the cardiac disease. Further studies are needed to investigate the true prevalence of PCD in the CHD patient population.

Exome-sequencing analysis recovered no mutations in PCD genes, cystic fibrosis transmembrane conductance regulator (CFTR), or other laterality associated genes, except for five patients (60 percent D-TGA) with mutations in multiple EGF-like domains-8 (*MEGF8*), a gene known to cause heterotaxy with TGA in mice (Table 9). Our whole-exome sequencing analysis of 52 TGA patients showed that none had homozygous or compound heterozygous novel or rare coding variants in the 30 known PCD genes, further indicating that the majority of TGA patients in our cohort do not have PCD. While we did recover heterozygous mutations in PCD genes in some TGA patients (similar to findings in heterotaxy patients), none were biallelic or homozygous. No mutations were recovered in any of the genes clinically associated with TGA, with the exception of mutations in *MEGF8*, seen in approximately 10 percent of our patients. Recessive *Megf8* mutations have been shown to cause heterotaxy with TGA in mice, as well as a subtype of Carpenter syndrome with left-right patterning defects, and TGA. In our cohort, four TGA patients had predicted damaging *MEGF8* mutations, with one patient likely harboring biallelic mutations consistent with a recessive disease model. However, this patient did not exhibit hallmarks of Carpenter's syndrome (craniocynostosis, digit and situs anomalies), but did have cleft lip and palate.

Together, these findings show that patients with D- and L-TGA exhibit high prevalence of abnormal CM and low nNO—with abnormal CM associated with increased respiratory symptoms. Our sequencing analysis suggests that *MEGF8* mutations may play a significant role in TGA pathogenesis. These findings reinforce the notion that a high prevalence of CD is associated with a wide spectrum of CHD. In addition, they suggest that isolated D- and L-TGA can arise from the disruption of left-right patterning. In conjunction with our outcome studies, these observations suggest that further studies are warranted to determine whether patients with TGA and other CHD undergoing surgical palliation may be at increased risk for postoperative complications related to airway CD.

**Table 8. Multivariate Regression Analysis of Respiratory Symptoms in TGA Patients**

Independent Variable	All Symptoms		PCD Symptoms		LRT Symptoms <sup>†</sup>		URT Symptoms <sup>‡</sup>	
	$\beta$	<i>P-value</i>	$\beta$	<i>P-value</i>	$\beta$	<i>P-value</i>	$\beta$	<i>P-value</i>
<b>Abnormal versus Normal C</b>	1.497	0.022	0.876	0.023	0.683	0.111	0.814	0.044
<b>Low versus Normal nNO</b>	0.801	0.208	0.524	0.166	0.117	0.781	0.686	0.087

\*PCD Symptoms <sup>†</sup>LRT Symptoms: Lower respiratory symptoms <sup>‡</sup>URT Symptoms: Upper respiratory symptoms

**Table 9. Novel *MEGF8* Coding Mutations Recovered in TGA Patients**

ID	Ethnicity	TGA Type	Ciliary Motion	Base Change	AA Change	Allele Frequency	Zygoty	PolyPhen2	SIFT	CADD
<b>7110</b>	White	D-TGA	Normal	7220G>A	R2407H	0.5%	HET	probably_damaging	deleterious	28.8
				7504G>A	V2502I	0.3%	HET	benign	tolerated	14.8
<b>7118</b>	White	L-TGA	Normal	2809C>T	R937W	0.1%	HET	probably_damaging	deleterious	18.6
<b>7138</b>	White	D-TGA	Normal	7472C>T	P2491L	0.7%	HET	benign	tolerated	0.005
<b>7152</b>	White	D-TGA	Abnormal	7573G>A	V2525M	0.1%	HET	possibly_damaging	deleterious	18.4
<b>7216</b>	White	L-TGA	Normal	700G>A	A234T	novel	HET	possibly_damaging	tolerated	18.0
				7220G>A	R2407H	0.5%	HET	probably_damaging	deleterious	28.8
				7504G>A	V2502I	0.3%	HET	benign	tolerated	14.8

Submitted for publication: ***DNAH6* and its interactions with PCD genes in heterotaxy and primary ciliary dyskinesia** (Li et al, 2015) (This article is not listed in Question 20 because PA Department of Health is not cited as a funding source.)

Heterotaxy, a birth defect involving left-right patterning defects, and PCD, a sinopulmonary disease with dyskinetic/immotile airway cilia, are seemingly disparate diseases. However, they have an overlapping genetic etiology involving mutations in cilia genes, a reflection of the common requirement for motile cilia in left-right patterning and airway clearance. While PCD is a monogenic recessive disorder, heterotaxy has a more complex, largely non-monogenic etiology. In this study, we show that mutations in the novel dynein gene *DNAH6* can cause heterotaxy and ciliary dysfunction similar to PCD. We provide the first evidence that trans-heterozygous interactions between *DNAH6* and other PCD genes can cause heterotaxy.

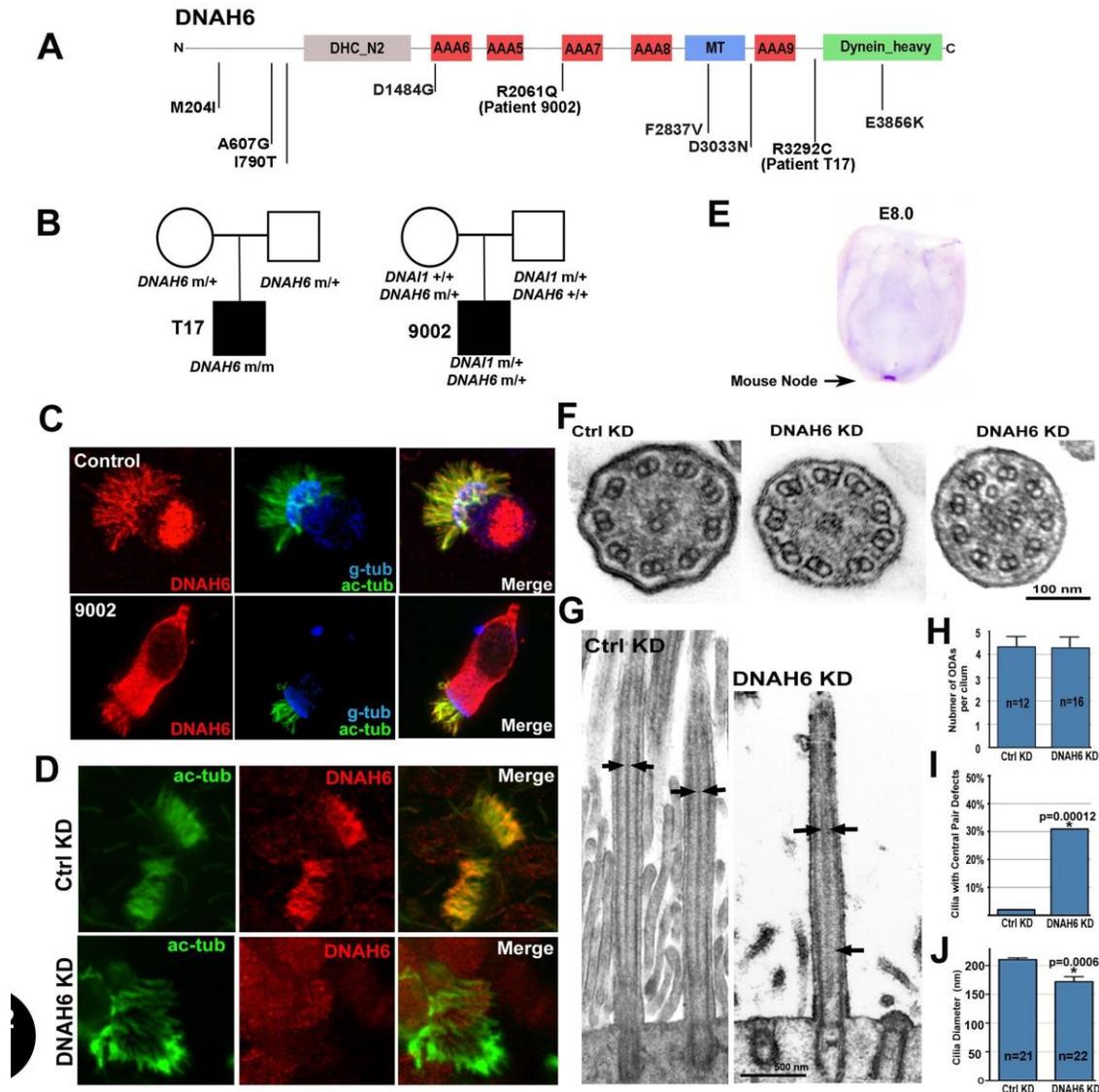
*DNAH6* was initially identified as a candidate heterotaxy/PCD gene by filtering exome-sequencing data from 25 heterotaxy patients stratified by whether they have airway motile cilia defects. A notable finding from the exome sequencing was a heterotaxy patient harboring a heterozygous *DNAH6* mutation together with a rare heterozygous PCD-causing *DNAI1* mutation, suggesting *DNAH6/DNAI1* trans-heterozygous interactions (Figure 4). In zebrafish, *dnah6* morpholino knockdown disrupted motile cilia in Kupffer's vesicle required for left-right patterning and caused heterotaxy with abnormal cardiac/gut looping (Figure 5). Similarly, *DNAH6* siRNA knockdown disrupted motile cilia in human and mouse respiratory epithelia (Figure 6).

Consistent with this observation, targeted resequencing of 149 additional heterotaxy patients using Ion Torrent amplicon resequencing showed that five of six patients with heterozygous *DNAH6* mutations also had heterozygous mutations in *DNAH5* or other PCD genes (Table 10). We functionally assayed for *DNAH6/DNAH5* and *DNAH6/DNAI1* trans-heterozygous interactions using subthreshold double-morpholino knockdown in zebrafish and showed that this process caused heterotaxy (Figure 6). Similarly, subthreshold siRNA knockdown of *Dnah6* in heterozygous *Dnah5* or *Dnai1* mutant mouse respiratory epithelia disrupted motile cilia function (Figure 6). Together, these findings show that *DNAH6* deficiency and trans-heterozygous interactions of *DNAH6* with other PCD genes can cause heterotaxy and airway ciliary dysfunction. These findings support an oligogenic disease model with broad relevance for further interrogating the complex genetic etiology of CHD and other human diseases.

An oligogenic model of disease for heterotaxy is compelling, given the complexity of ciliogenesis. Furthermore, this model is consistent with clinical studies showing that heterotaxy has a largely non-monogenic etiology. Our analysis focused on assaying the effects of deficiency or haploinsufficiency. This focus is appropriate since most PCD mutations are, in fact, loss of function like the *DNAI1* mutation found in patient 9002. While analysis of *DNAH6* missense mutations would have been worthwhile, the very large size of the *DNAH6* transcript (12,477 base pairs) precluded the pursuit of such experiments. An oligogenic model of disease is yet to be demonstrated clinically for heterotaxy or PCD. But PCD patients with only a single heterozygous pathogenic PCD mutation, reminiscent of the pathogenic *DNAI1* mutation found in heterotaxy patient 9002, have been reported by others and also seen in our cohort of participants. These findings suggest the possibility that PCD may also arise in an oligogenic context with trans-

heterozygous PCD mutations. An oligogenic disease model has been suggested previously for other ciliopathies. For example, cilia genes *CEP290*, *RPGRIP1L*, *AH11*, and *KIF7* have been reported to act as genetic modifiers in various ciliopathies.

In conclusion, functional analysis of airway ciliary motion in heterotaxy patients identified the novel dynein, *DNAH6*, as a candidate gene for heterotaxy and PCD. Our experimental modeling shows for the first time that *DNAH6* is essential for motile cilia function mediating left-right patterning and in mucociliary airway clearance. Our findings suggest that *DNAH6* can act in a recessive manner and also support a more complex oligogenic model of disease involving trans-heterozygous interactions with other PCD mutations. While we only interrogated for digenic interactions between *DNAH6* and other dyneins, more complex genetic interactions should be further investigated in future studies. Overall, these findings have broad relevance for interrogating the complex genetics of heterotaxy and other ciliopathies. More insights into the genetic etiology of heterotaxy may have clinical translational application in providing better patient stratification to identify those at risk for post-surgical respiratory complications related to airway ciliary dysfunction. This method can help optimize the clinical care of high-risk heterotaxy/CHD patients to help improve overall prognosis.



### Figure 4. *DNAH6* Mutations and the Role of *DNAH6* in Motile Cilia Function

(A) Schematic of *DNAH6* protein structure; predicted functional domains are shown together with the position of novel and rare mutations identified in heterotaxy patients.

(B) Pedigrees show heritable transmission of homozygous *DNAH6* mutation in patient T17 and double heterozygous *DNAI1/DNAH6* mutations in heterotaxy patient 9002.

(C) *DNAH6* antibody (red) is localized in the ciliary axoneme (green) in control human respiratory epithelium and in patient 9002.

(D) *DNAH6* (red) antibody staining is lost in the ciliary axoneme (green) with sh*DNAH6* knockdown of human respiratory epithelia.

(E) Whole-mount *in situ* hybridization analysis showed *Dnah6* is exclusively expressed in the embryonic node of E8.0 mouse embryo.

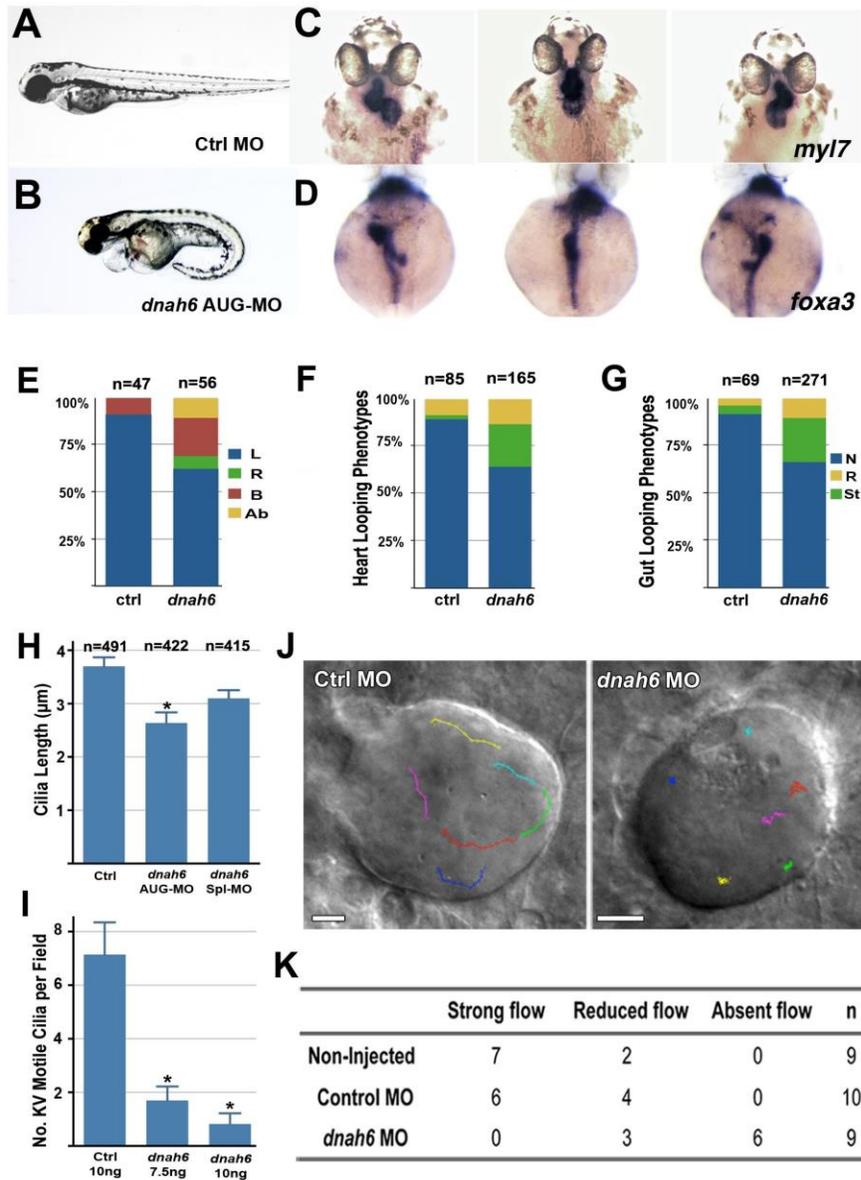
(F-J) Electron microscopy of human airway epithelia after *DNAH6* knockdown showed missing central pair and extra microtubules versus 9+2 cilia ultrastructure in normal airway cilia (F). This also can be observed in longitudinal views (arrows in G). Quantitation using EM cross sections showed no ODA defects (H), confirmed the central pair defects (I, Chi-square test, p-value =  $1.2 \times 10^{-4}$ ), and also showed reduction in cilia diameter (J, two-tailed Student t-test, p-value = 0.0006).

**Table 10. *DNAH6* and PCD Gene Mutations Identified in Heterotaxy Patients**

Patient	Race	Sex	Cilia Function <sup>‡</sup>	Gene	Nucleotide Change	Protein Change	Zygoty	Allele Freq*
<b>Children's National Medical Center</b>								
9002	White	M	<i>CD</i>	<i>DNAH6</i>	IVS1+2_3insT	Truncation	het	0.048%
				<i>DNAH6</i>	c.6182G>A	p.R2061Q	het	Novel
9027	Hispanic	F	<i>CD</i>	<i>DNAH6</i>	c.4451A>G	p.D1484G	het	Novel
<b>Cincinnati Children's Hospital</b>								
T17	White	M	<i>n.d.</i>	<i>DNAH6</i>	c.C9874T	p.R3292C	homo	Novel
<b>Tokyo Women's Medical University</b>								
JP2090	Asian	F	<i>n.d.</i>	<i>DNAH6</i>	c.G11566A	p.E3856K	het	Novel
				<i>DNAH5</i>	c.A737G	p.N246S	het	Novel
JP2637	Asian	F	<i>n.d.</i>	<i>DNAH6</i>	c.G612A	p.M204I	het	Novel
JP3617	Asian	F	<i>n.d.</i>	<i>DNAH6</i>	c.G9097A	p.D3033N	het	Novel
				<i>DNAH5</i>	c.C12472T	p.R4158W	het	0.007%
JP3634	Asian	M	<i>n.d.</i>	<i>DNAH6</i>	c.C1820G	p.A607G	het	Novel
				<i>DNAH5</i>	c.C12472T	p.R4158W	het	0.007%
<b>Children's Hospital of Philadelphia</b>								
GOLD53	White	F	<i>n.d.</i>	<i>DNAH6</i>	c.T2369C	p.I790T	het	0.06%
				<i>DNAH5</i>	c.C3514A	p.Q1172K	het	0.2%
				<i>RSPH4A</i>	c.C1990T	p.P664S	het	0.001%
GOLD54	Black	M	<i>n.d.</i>	<i>DNAH6</i>	c.T8509G	p.F2837V	het	0.01%
				<i>DNAH11</i>	c.G13135T	p.G4389C	het	Novel
				<i>LRRC50</i>	c.C1582G	p.T531R	het	Novel

<sup>†</sup> Highlighted changes in grey are known pathogenic PCD causing mutations

<sup>‡</sup> *CD*: airway ciliary dysfunction as determined by the finding of low nasal nitric oxide and abnormal airway ciliary motion observed by videomicroscopy \* Allele frequencies for rare variants derived from NHLBI exome database



**Figure 5: *dnah6* morpholino knockdown in zebrafish embryo causes laterality defects**

(A,B) *dnah6* MO injected embryos exhibited curly tail and cardiac edema phenotype at 48 hours post fertilization (hpf).

(C,D) *dnah6* MO injected embryos at 48 hpf exhibited heart (C) and gut (D) looping defects, including failure to loop (middle panels) and reversal looping (right panels).

(E) *In situ* hybridization analysis revealed abnormal right-sided (R), bilateral (B), and absent (Ab) *spaw* expression after *dnah6* MO knockdown.

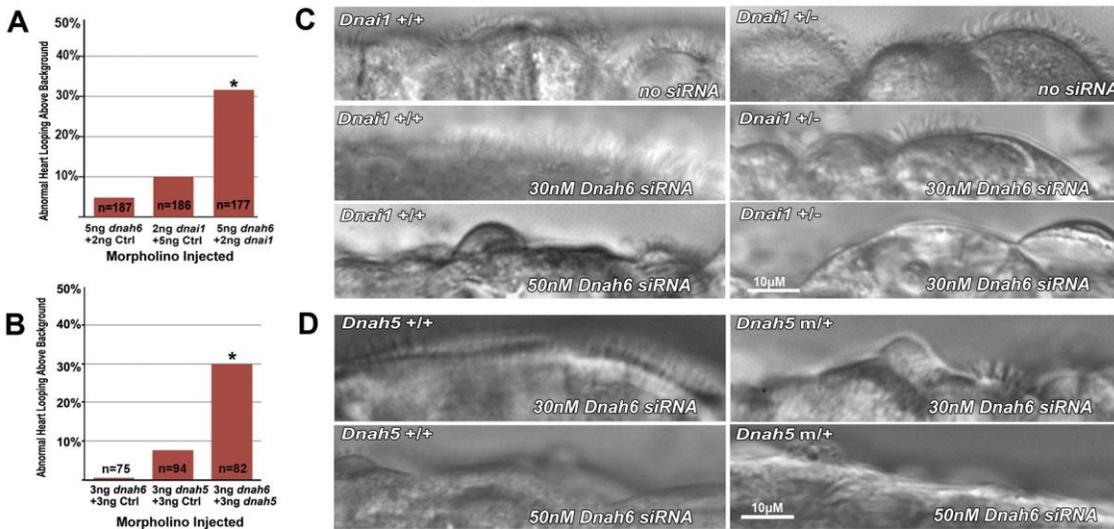
(F,G) Heart/gut looping defects were observed in *dnah6* MO-injected embryos, including normal (N), right-sided (R), and straight (St) heart/gut looping phenotypes.

(H). KV cilia were shorter (middle) in *dnah6* AUG-MO but not *dnah6* spl-MO-injected embryos (p-value=0.0017).

(I). High-speed videomicroscopy showed reduction in KV motile cilia of *dnah6* MO injected embryos (p=  $1.6 \times 10^{-4}$  for 7.5 ng and p=  $1.1 \times 10^{-5}$  for 10 ng *dnah6* MO; two-tailed Student's t-test).

(J) Little bead movement (color tracing) was observed in *dnah6* MO-injected embryos.

(K) KV flow was absent or reduced with *dnah6* knockdown (Chi-square test, p-value = 0.0192).



### Figure 6: *Dnah6* genetically interacts with *Dnai1* and *Dnah5* to cause heterotaxy and PCD

(A, B) Embryos injected with subthreshold dose of *dnah6* and *dnai1* MO show increased heart-looping defects compared with Ctrl MO injections ( $n=177$ ,  $p\text{-value}=3.8\times 10^{-8}$ ), or single injection of either *dnai1* ( $p=9.29\times 10^{-9}$ ) or *dnah6* ( $p=2.04\times 10^{-8}$ ) MO at the same MO dose (A). Similar results were observed with subthreshold *dnah5/dnah6* double MO knockdown ( $n=82$ ;  $p=1.74\times 10^{-5}$ , Bonferroni corrected).

(C, D) Reciliating mouse airway epithelia from wildtype (+/+) and heterozygous (+/-) *Dnai1* knockout (C) or *Dnah5* mutant (D) mice show robust ciliation and ciliary motion (Movie S5, S6). 30nM *Dnah6* siRNA had no effect on ciliation or cilia motility in wildtype airway epithelia; but in heterozygous *Dnai1* or *Dnah5* mutant airway, ciliation was reduced and ciliary motion was dyskinetic (Movie S5, S6). With 50nM siRNA, little or no cilia were seen in wildtype and heterozygous *Dnai1* or *Dnah5* mutant mouse airway.

Submitted for publication: **Respiratory motile cilia dysfunction in cranioectodermal dysplasia patient with biallelic *WDR35* mutations.** (Li et al, 2015)

Among participants recruited into our study is a patient diagnosed with cranioectodermal dysplasia (CED), also known as Sensenbrenner syndrome. This is a rare genetic disorder in which patients exhibit pleomorphic clinical features such as narrow thorax, dolichocephaly, developmental delay, liver fibrosis, and chronic renal failure. Of greatest clinical concern for CED patients is respiratory distress due to rib cage narrowing caused by skeletal dysplasia. This condition is frequently lethal in the neonatal period, making stabilization and support of respiratory function a critical priority. While surgical treatment advances for thoracic hypoplasia offer hope to children suffering from CED, prognosis remains poor.

CED is a genetically heterogeneous recessive disorder with phenotypic/genetic overlap. Currently >12 genes are known to cause rib cage dysplasia. Among our study patients is an individual with CED whom we identified (by whole-exome sequencing analysis) as having biallelic *WDR35* mutations (Figure 7). In contrast to PCD, CED is thought to mainly affect primary cilia function. However, this patient was notable in exhibiting severe respiratory complications despite having very mild thoracic dystrophy that did not require surgical palliation. Given that many proteins found in primary cilia are also found in motile cilia, we hypothesize that this individual may suffer respiratory complications related to an essential requirement for *WDR35* in motile cilia function. Respiratory ciliary dysfunction in this patient may go unappreciated, given that respiratory complications would be attributed to the thoracic dystrophy associated with CED.

Using high-resolution videomicroscopy of nasal epithelial biopsy, we showed that this patient had respiratory ciliary dysfunction characterized by immotile/dyskinetic cilia. At the same time, assessment showed abnormally low nNO—both characteristics typically seen with PCD. While this patient exhibited relatively mild thoracic dystrophy not requiring surgical remediation, there was, nevertheless, newborn respiratory distress, restrictive airway disease with possible obstructive airway involvement (Figure 8; Table 11), repeated respiratory infection, and atelectasis, a sinopulmonary disease associated with mucociliary clearance defects due to motile cilia dysfunction in the airway (Table 11). Exome sequencing analysis of this patient identified compound heterozygous mutations in *WDR35* but no mutations in any of the 30 known PCD genes or other cilia-related genes. Given that *WDR35* is only known to be required for primary cilia function, we carried out *WDR35* siRNA knockdown in human respiratory epithelia to assess the role of *WDR35* in motile cilia function. This experiment showed that *WDR35* deficiency disrupted ciliogenesis in the airway, indicating that *WDR35* is also required for motile cilia formation.

Together, these findings demonstrate that patients with *WDR35* mutations can have airway mucociliary clearance defects masked by the restrictive airway disease of asphyxiating thoracic dystrophy. These observations further suggest that ciliopathy patients with rib cage dysplasia who exhibit sequelae of respiratory complications warrant further clinical evaluation for respiratory ciliary dysfunction. Early diagnosis of respiratory CD may allow early intervention with aggressive pulmonary therapy that can help improve the long-term prognosis for these patients, who have a lifelong struggle with a plethora of critical illnesses.

**Figure 7. Biallelic *WDR35* mutations in CED patient**

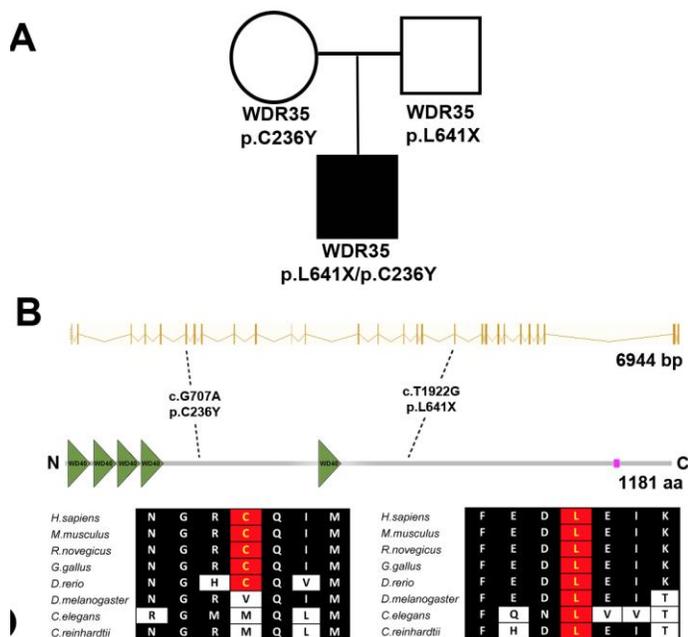


Figure 7. (A) Exome sequencing analysis recovered two biallelic *WDR35* mutations with p.C236Y maternally inherited and p.L641X paternally inherited. (B) Positions of the *WDR35* mutations in the protein and the cross-species conservation.

**Figure 8. Pulmonary function tests showed restrictive airway disease.**

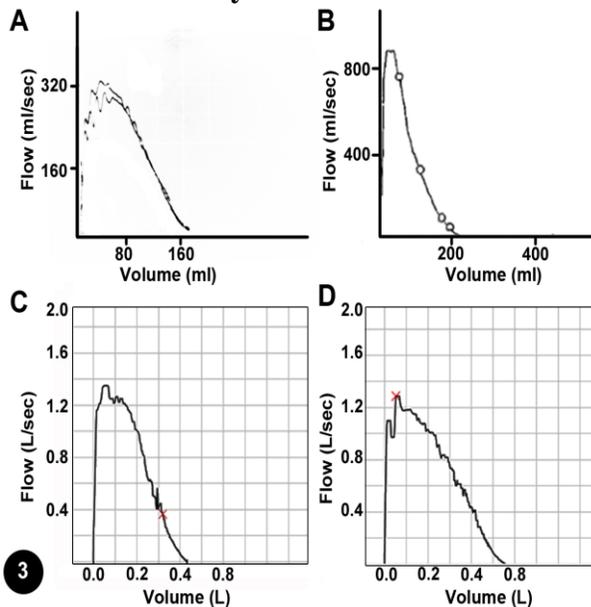


Figure 8. Forced deflation spirometry at seven months (A) demonstrated mild restrictive airway defect (FVC 78% predicted). Raised volume rapid thoracic compression spirometry at 3 years (B) showed restrictive (FVC 48%) and obstructive (FEF<sub>75</sub> 37%) respiratory defects. At 5 (C) and 6 (D) years, forced deflation spirometry demonstrated predominantly restrictive defect (FVC 56% and 61%), which improved compared to 3 years of age (B).

**TABLE 11. Pulmonary Function Assessments**

Parameters <sup>†</sup>	5/30/07 <sup>a</sup>	11/10/09 <sup>b</sup>	2/28/12 <sup>a</sup>	1/2/13 <sup>a</sup>
Age	7 mos	3 yrs	5 yrs	6 yrs
FVC	78%	48%	56%	61%
FEV <sub>0.5</sub> or FEV <sub>1</sub>		45%	57% <sup>†</sup>	60% <sup>†</sup>
FEF <sub>75</sub> or FEF <sub>25-75</sub>	61% <sup>††</sup>	37%	116%	88%
TLC		47%		

<sup>a</sup>Measurement obtained using forced-deflation technique <sup>b</sup>Measurement obtained using raised-volume rapid thoracic compression technique

<sup>†</sup>FVC: forced vital capacity; FEV: forced expiratory volume; FEF: forced expiratory flow; TLC: total lung capacity.

**Meeting Abstracts and Presentations:**

Li, Y., Yagi, H., Onuoha, E.O., Barmada, M., Tsang, M., and Lo, C.W. (2011). Multigenic Etiology of CHD With Heterotaxy Involving PCD and Cilia Genes . Circulation 124:A16678.

Li, Y., Yagi, H., Onuoha, E., Pazour, G., Leatherbury, L., Tsang, M., Lo, C.W. (2012). NAT10 Mutation Causes Ciliary Aplasia and Congenital Heart Disease Associated with Heterotaxy. Circulation. 126 : A19282.

Garrod, A., Zahid , M., Francis, R., Khalifa, O., Devine, W., Leatherbury, L., and C.W. Lo (2013). Airway ciliary dysfunction and increased sinopulmonary symptoms in congenital heart disease patients with and without heterotaxy. Resp Crit Care Med. A96. Platform presentation at the 2013 American Thoracic Society.

Li, Y., Yagi, H., Onuoha E., Pazour, G., Leatherbury, L., Tsang, M., Lo, C.W. (2013). DNAH6 and Digenic Interactions with DNAI1 and DNAH5 Modulate Human Respiratory Cilia motility and Left-Right Body Asymmetry Presented at FASEB Scientific Conference “Biology of Cilia and Flagella”

Zahid M, Khalifa O, Devine W, Yau C, Francis R, Lee DM, Tobita K, Wearden P, Leatherbury L, Webber S, Lo CW. (2012). Airway Ciliary Dysfunction in Patients with Transposition of the Great Arteries. Circulation. 126:A15746.

Zahid, M. Li, Y., Khalifa, O., Devine, W., Yau, C., Francis, R., Lee, D., Tobita, K., Wearden, P., Leatherbury, L., Webber, S., Lo, C.W. (2013). High Prevalence of Respiratory Ciliary Dysfunction Associated with Transposition of the Great Arteries. Presented at FASEB Scientific Conference “Biology of Cilia and Flagella”.

Zahid, M. (2014). Cilia and Congenital Heart Disease: What is the Connection? Journal of Heart Disease 2014:11(1):120.

Zahid M, Li, Y, Tian X, Francis R, Kena N, Khalifa O, Devine W, lee D M, Yau C, Lemke K, Leatherbury L, Tobita K, Lo C. (2014). High Prevalence of Respiratory Ciliary Dysfunction Associated with transposition of the Great Arteries. Journal of the American College of Cardiology 63(12):A123.

**18. Extent of Clinical Activities Initiated and Completed.** Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be “No.”

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

       Yes  
  X   No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

Yes

No

**If “Yes” to either 18(A) or 18(B), items 18(C) – (F) must also be completed.** (Do NOT complete 18(C-F) if 18(A) and 18(B) are both “No.”)

18(C) How many hospital and health care professionals were involved in the research project?

Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

Number of subjects originally targeted to be included in the study

Number of subjects enrolled in the study

**Note:** Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:

Males

Females

Unknown

Ethnicity:

Latinos or Hispanics

Not Latinos or Hispanics

Unknown

Race:

American Indian or Alaska Native

Asian

Blacks or African American

Native Hawaiian or Other Pacific Islander

White

Other, specify: \_\_\_\_\_

Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

**19. Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?

Yes  
 No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

Yes  
 No

19(C) Please describe how this project involved human embryonic stem cells:

**20. Articles Submitted to Peer-Reviewed Publications.**

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

- Project 01 – Smith – Three cases of isolated
- Project 01 – Smith – Investigation of NEB1 deletions
- Project 03 – Zhang – Molecular profiling of aromatase
- Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

**Note:** The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed

acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. High prevalence of respiratory ciliary dysfunction in heterotaxy patients with congenital heart disease	Nakhleh, N., Swisher, M., Francis R., Giese, R., Chatterjee, B., Connelly, P., Sami, I., Kuehl, K., Olivier, K., Jonas, R., Tian, X., Zariwala, M., Omran, H., Leigh, M., Knowles, M., Leatherbury, L., and Lo, C.W.	Circulation	November 2011	<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input checked="" type="checkbox"/> Published
2. Airway ciliary dysfunction and sinopulmonary symptoms in congenital heart disease patients	Garrod, A.S., Zahid, M., Tian, X., Francis, R.J., Khalifa, O., Devine W. Beerman, L., Gabriel, G., Leatherbury, L., and Lo, C.W.	Annals Am Thoracic Society	May 2014	<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input checked="" type="checkbox"/> Published
3. Respiratory motile cilia dysfunction in cranioectodermal dysplasia patient with biallelic <i>WDR35</i> mutations	Li Y, Garrod AS, Madan-Khetarpal S, Sreedher G, McGuire M, Yagi H, Klena NT, Gabriel GC, Khalifa O, Zahid M, Panigraphy A, Weiner DJ, Lo CW.	Am J of Medical Genetics	November 2014	<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input checked="" type="checkbox"/> Published
5. Airway Cilia Dysfunction and Respiratory Symptoms in Patients with Transposition of the Great Arteries	Zahid M., Li, You, L., Tian, X., Francis, R., Klena, K., Devine, W., Lee, D.M., Yau, C., Lemke, K., Beerman, L., Munoz, R., Wearden, P. Tobita, K., Leatherbury, L., Khalifa, O., Lo, C.W.	European J of Cardiology	December 2014	<input checked="" type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> Published
6. Airway Ciliary Dysfunction and Postoperative Complications in Congenital Heart Disease Patients	Stewart, E., Zahid, M., Adams, P., Khalifa, O., Feingold, B., Devine, W., Leatherbury, L., Munoz, R., Wearden, P., and Lo, C.W.	Circulation	December 2014	<input checked="" type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes \_\_\_\_\_ X \_\_\_\_\_ No \_\_\_\_\_

If yes, please describe your plans:

We are conducting validation experiments on the ciliome gene *NATIO*, another gene for which homozygous mutations were recovered in a heterotaxy patient. We will be putting this work together for publication in the coming months, as mouse models are generated to show the role of this gene in ciliogenesis and CHD pathogenesis.

**21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.**

Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert “None”; do not use “Not applicable.” Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Our study has future clinical translational possibilities by providing insights into the association of airway ciliary dysfunction and the burden of ciliome mutations in respiratory complications and pulmonary morbidity in CHD patients. The findings from the current study provide the foundation for expanded interrogation of the role of ciliome mutations and respiratory ciliary dysfunction in increased pulmonary morbidity in CHD patients. The validation of these observations in further clinical studies will suggest the possibility of using genetic testing for ciliome mutations to predict CHD patients at risk for respiratory complications, thereby allowing interventions with pre- and postoperative pulmonary therapies to help enhance airway clearance and improve outcome for CHD patients, who often must undergo repeated high-risk cardiac operations.

**22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment.** Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert “None”; do not use “Not applicable.” Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

**23. Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes \_\_\_\_\_ No X

If “Yes” to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is “No.”)

- a. Title of Invention:
- b. Name of Inventor(s):
- c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):
- d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?  
Yes \_\_\_\_\_ No \_\_\_\_\_

If yes, indicate date patent was filed:

- e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?  
Yes \_\_\_\_\_ No \_\_\_\_\_  
If yes, indicate number of patent, title and date issued:  
Patent number:  
Title of patent:  
Date issued:

- f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes \_\_\_\_\_ No \_\_\_\_\_

If yes, how many licenses were granted? \_\_\_\_\_

- g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes \_\_\_ No \_\_\_

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes \_\_\_\_\_ No X

If yes, please describe your plans:

**24. Key Investigator Qualifications.** Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

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## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

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NAME Cecilia W. Lo	POSITION TITLE Professor		
eRA COMMONS USER NAME (agency login) cecilialo			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
Mass. Institute of Technology	B.S.	05/74	molecular biology
Rockefeller University	Ph.D.	06/79	cell/developmental biology
Harvard Medical School	Postdoc Training	79-80	molecular biology

### A. Personal Statement

The main research interest in my laboratory are focused on elucidating the genetic causes and developmental mechanisms of human congenital heart disease (CHD), one of the most common birth defects in the human population. These studies entail a combination of studies involving animal modeling with mice and zebra fish and the integration of findings from the mouse models with human clinical studies. These studies have led to the realization that mutations affecting cilia structure and function play an important role in congenital heart disease. As one of the 4 funded CvDC centers, we have been engaged in a 5 year ultrasound screen to interrogate the genetic etiology of CHD. We have ultrasound scanned ~100,000 fetuses from >3000 pedigrees for >10 fold genome coverage. Over 250 mutant lines have been recovered with a wide spectrum of CHD, all of which have been cryopreserved at the Jackson Laboratory for public access and curated in the Mouse Genome Informatics Database (type "CvDC" in Quick Search Mouse in <http://www.informatics.jax.org> will retrieve all our mouse mutants). Using whole mouse exome sequencing analysis, we have recovered the pathogenic mutations in 134 mutant lines encompassing 90 genes. Very striking is the recovery of many genes encoding proteins required for motile cilia function, including cilia genes encoding proteins required for non-motile or primary cilia structure and function in mutants with congenital heart disease without laterality defects. Together these findings suggest the cilia plays a central role in CHD pathogenesis, providing the basis for our human clinical studies focused on interrogating for ciliome mutation in patients with CHD.

Our first clinical studies involving CHD patients with heterotaxy showed a surprising 42% incidence of ciliary dysfunction in the airway. This is characterized by reduced levels of nasal nitric oxide and ciliary motion defects, data obtained from the analysis of nasal respiratory epithelia obtained from nasal samplings. These same changes are also observed in patients with primary ciliary dyskinesia (PCD). Using exome sequence capture and next generation sequencing of 48 patients, including 8 PCD patients as disease controls and 13 health controls, we have shown CHD patients with CD are enriched for mutations in genes known to cause PCD, and are indistinguishable from PCD patients. Moreover, the CHD patients with airway CD also showed significantly more respiratory symptoms and disease. Together these findings strongly suggest a common genetic etiology for PCD and CHD associated with heterotaxy. We have now expanded these studies to over 200 patients with CHD without heterotaxy, and showed a similar high prevalence of ciliary dysfunction, indicating ciliary dysfunction have a broader role to play in CHD pathogenesis. Moreover, we have conducted postsurgical outcome analysis, which showed ciliary dysfunction is highly correlated with increase in postsurgical respiratory complications, indicating the ciliary dysfunction have significant impact on clinical outcomes. These findings suggest identification of CHD patients with CD can provide the means for therapeutic intervention pre and postsurgically to reduce pulmonary morbidity. Overall, through these studies, we hope to determine whether the cilium may comprise a central disease pathway in human CHD pathogenesis. Such findings will pave the way for clinical translational studies to improve the standard of care for CHD patients.

This may include diagnostic testing for CD and providing pulmonary therapy to help improve outcome.

## **B. Positions and Honors**

### **PRESENT AND PREVIOUS POSITIONS**

6/2011 to present F Sargent Cheever Chair, University of Pittsburgh Department of Developmental Biology  
8/2009 to present Chairman and Professor, Department of Developmental Biology,  
University of Pittsburgh School of Medicine  
7/2014 to present Executive Director of the Integrative Systems Biology Graduate Program  
9/2004 to 7/2009 Director, Genetics and Developmental Biology Center, NHLBI/NIH  
9/2001 to 7/2009 Lab Chief, Laboratory of Developmental Biology, NHLBI/NIH  
7/1980 to 8/2001 Assistant, Associate, Full Professor, Biology Department, University of Pennsylvania

### **C. Selected Peer Reviewed Publications (2012-present)**

- Li, Y, Klena, N.T., Gabriel, G.C., Liu, X., Kim, A.J., Lemke, K., Chen, Y., Chatterjee, B., Damerla, R.R., Chang, C.F., Yagi, H., San Augustin, J.T., Thahir, M., Anderton, S., Lawhead, C., Vescovi, A., Pratt, H., Morgan, J., Haynes, L., Smith, C.L., Eppig, J.T., Reinholdt, L., Francis, R., Leatherbury, L., Ganapathiraju, M., Tobita, K., Paozur, G.J., Lo, C.W. (2015). Global genetic analysis in mice unveils central role for cilia in congenital heart disease. *Nature*. In press.
- Eguether, T., San Augustin, J., Keady, B.T., Jonassen, J.A., Liang, Y., Francis, R., Tobita, K., Johnson, C.A., Abdelhamed, Z.A., Lo, C.W., Pazour, G.J. 2014. IFT27 links the BBSome to IFT for maintenance of ciliary signaling compartment. *Dev Cell*. In press.
- Czarnecki, P, Gabriel, G, Manning, D., Sergeev, M., Lemke, K, Klena, K., Liu, X., Chen, Y., Li, Y., San Augustin, J, Garnaas, M., Francis, R, Tobita, K., Goessling, W., Pazour, Gregory, Lo, C.W., D. Beier, and J. Shah (2014) ANKS6 is the critical activator of NEK8 kinase, regulating situs determination, cardiopulmonary and renal development" *Nature Communications*, In press.
- Garrod, A.S., Zahid, M., Tian, X., Francis, R.J., Khalifa, O., Devine W. Beerman, L., Gabriel, G., Leatherbury, L., and Lo, C.W. (2014). Airway ciliary dysfunction and sinopulmonary symptoms in congenital heart disease patients. *Annals American Thoracic Society* 11:1426-1432.
- Liu, X.Q., Francis, R., Kim, A.J., Ramirez, R., Chen, Y., Subramanian, R., Anderton, S., Kim, Y., Wong, L., Morgan, J., Pratt, H., Reinholdt, L., Devine, W., Leatherbury, L., Tobita, K., and Lo, C.W. (2014). Interrogating congenital heart defects with noninvasive fetal echocardiography in a mouse forward genetic screen. *Circulation: Cardiovascular Imaging*. 7(1):31-42. PMID: 3962690.
- Hjeij, R. Onoufriadis, A. Watson, C.M., Slagle, C.E., Klena, N.K., Dougherty, G.W., Kurkowiak, M. Loges, N.T., Diggle, C.P., Morante, N.F.C., Gabriel, G.C. Lemke, K.L., Li, Y. Pennekamp, P., Menchen, T., Konert, F., Martin, K., Mans, D.A., Letteboer, S.J.F., Werner, C., Burgoyne, T., Westermann, C., Rutman, A., Carr, I.M., O'Callaghan, C., Moya, E., Chung, E.M.K., UK10K, Sheridan, E., Nielsen, K.G., Roepman, R. Bartscherer, K., Burdine, R.D., Lo, C.W., Omran, H., and Mitchison, H.M. (2014). CCDC151 mutations cause primary ciliary dyskinesia by disruption of the outer dynein arm docking complex formation. *Am J Human Genet*. 95:257-74.
- Tarkar A, Loges NT, Slagle CE, Francis R, Dougherty GW, Tamayo JV, Shook B, Cantino M, Schwartz D, Jahnke C, Olbrich H, Werner C, Raidt J, Pennekamp P, Abouhamed M, Hjeij R, Köhler G, Griese M, Li Y, Lemke K, Klena N, Liu X, Gabriel G, Tobita K, Jaspers M, Morgan LC, Shapiro AJ, Letteboer SJ, Mans DA, Carson JL, Leigh MW, Wolf WE, Chen S, Lucas JS, Onoufriadis A, Plagnol V, Schmidts M, Boldt K; UK10K, Roepman R, Zariwala MA, Lo CW, Mitchison HM, Knowles MR, Burdine RD, Loturco JJ, Omran H. Dyl1c1 is required for axonemal dynein assembly and ciliary motility. (2013). *Nature Genet*.45:995–1003
- Cui, C.C., Chatterjee, B., Lozito, T., Zhang, Z., Francis, R.J., Yagi, H., Antosnewski, L.M., Sanker, S., Francis, D., Yu, Q., San Augustin, J., Puligilla, C., Kelley, M.W., Spilliotis, E.T., Kwiatkowski, A., Pazour, G.J., Hukriede, N.A., and Lo, C.W. (2013). Wdpcp, a PCP protein required for ciliogenesis, regulates directional cell migration and cell polarity by direct modulation of the actin cytoskeleton. *PLOS Biology* 11(11): e1001720. doi:10.1371. PMID: 3841097
- Nakhleh, N., Swisher, M., Francis R., Giese, R., Chatterjee, B., Connelly, P., Sami, I., Kuehl, K., Olivier, K., Jonas, R., Tian, X., Zariwala, M., Omran, H., Leigh, M., Knowles, M., Leatherbury, L., and Lo, C.W. (2012). High prevalence of respiratory ciliary dysfunction in heterotaxy patients with congenital heart disease. *Circulation* 125:2232-2242. PMID: 22499950
- Keady, B.T., Ssammani, R., Tobita, K., Tsuchya, M., San Augustin, J.T., Follit, J.A., Jonassen, J.A., Subramanian, R. Lo, C.W., and Pazour, G.J. (2012). IFT25 links the signal-dependent movement of hedgehog components to intraflagellar transport. *Dev. Cell* 22: 940-951