On-line Supplement:

Primary Ciliary Dyskinesia: Longitudinal Study of Lung Disease by Ultrastructure Defect and Genotype

Stephanie D. Davis, Margaret Rosenfeld, Hye-Seung Lee, Thomas W. Ferkol, Scott D. Sagel, Sharon D. Dell, Carlos Milla, Jessica E. Pittman, Adam J. Shapiro, Kelli M. Sullivan, Keith R. Nykamp, Jeffrey P. Krischer, Maimoona A. Zariwala, Michael R. Knowles, Margaret W. Leigh and the Genetic Disorders of Mucociliary Clearance Consortium

Methods:

Genetic testing analysis:

All participants with “negative” genetics were tested with a genetic panel that included at least 28 genes associated with primary ciliary dyskinesia (PCD) and did not harbor two pathogenic mutations.

Criteria used to deem private alleles (either missense or in-frame alleles) as likely disease-associated included: a) loss-of-function variant identified along with missense or in-frame variant; b) clinical diagnosis of PCD, including congruent ciliary ultrastructural defects; c) rare variant (< 0.1 allele frequency in public databases such as dbSNP, ExAC, gnomAD and 1000Genomes); d) variant considered "disease-causing" by Mutation Taster algorithm (http://neurocore.charite.de/MutationTaster/); e) variant conserved across the species.

Additional Results

Table E1: Specific mutations in PCD genes

Participant	Gene name	Variant 1: base change (predicted protein change)	Variant 2: base change (predicted protein change)	In prior (Ref #) or current study only
ODA Group (n=55)				
ODA01	*ARMC4*	c.1969C>T (p.Gln657*)	c.1969C>T (p.Gln657*)	1
ODA02	*CCDC114*	c.742G>A (p.A248Sfs*52)	c.1391+5G>A (p.Ser432Argfs*7)	2
ODA03	*CCDC114*	c.337C>T (p.Arg113*)	c.337C>T (p.Arg113*)	Current
ODA04	*DNAH5*	c.3905delT (p.Leu1302Argfs*19)	c.6249G>A [p.Cys1962Ilefs*14; p.Gly2021Glufs*12]	Current
ODA05	*DNAH5*	c.4237C>T (p.Gln1413*)	1.6-kb deletion including exon 2 (p.?)	3
ODA06	*DNAH5*	c.832delG (p.Ala278Argfs*27)	c.1627C>T (p.Gln543*)	4
ODA07	*DNAH5*	c.12910-1G>C (p.?)	c.12910-1G>C (p.?)	5
ODA08	*DNAH5*	c.12910-1G>C (p.?)	c.12910-1G>C (p.?)	5
ODA09	*DNAH5*	c.2772delC (p.Leu925*)	c.6335_6336insT (p.Gln2112Hisfs*10)	Current
ODA10	*DNAH5*	c.13458dupT (p.Asn4487*)	deletion including exon 62 (p.?)	6
ODA11	*DNAH5*	c.9427A>T (p.Lys3143*)	c.10615C>T (p.Arg3539Cys)	7
ODA12	*DNAH5*	c.9427A>T (p.Lys3143*)	c.10615C>T (p.Arg3539Cys)	7
ODA13	*DNAH5*	c.832delG (p.Ala278Argfs*27)	c.5710-2A>G (p.?)	Current
ODA14	*DNAH5*	c.6988+2T>C (p.?)	c.7502G>C (p.Arg2501Pro)	Current
ODA15	*DNAH5*	c.6988+2T>C (p.?)	c.7502G>C (p.Arg2501Pro)	Current
ODA16	*DNAH5*	c.5114+1G>C (p.?)	c.8147T>C (p.Ile2716Thr)[§]	Current
ODA17	*DNAH5*	c.5983C>T (p.Arg1995*)	c.13760A>G (p.Tyr4587Cys)	Current
ODA18	*DNAH5*	c.1731-2A>T (IVS13-2A>T) (p.?)	c.4117-2A>G (p.?)	Current
ODA19	*DNAH5*	c.5983C>T (p.Arg1995*)	c.12009G>A (p.Trp4003*)	Current
ODA20	*DNAH5*	c.4348C>T (p.Gln1450*)	c.4348C>T (p.Gln1450*)	8
ODA21	*DNAH5*	c.12107G>A (p.W4036*)	c.4348C>T (p.Gln1450*)	Current
ODA22	*DNAH5*	c.10815delT (p.Pro3606Hisfs*23)	c.10815delT (p.Pro3606Hisfs*23)	Current
ODA23	*DNAH5*	c.7915C>T (p.Arg2639*)	c.8497C>T (p.Arg2833Cys)	Current
ODA24	*DNAH5*	c.9799C>T (p.Gln3267*)	c.5147G>C (p.Arg1716Pro)	Current
ODA25	*DNAH5*	c.1432C>T (p.Arg478*)	c.6932_6935delACTG (p.Asp2311Glyfs*14)	Current
ODA26	*DNAH5*	c.8141delA (p.Asn2714Metfs*30)	c.13760A>G (p.Tyr4587Cys)	Current
ODA27	*DNAH5*	c.5588delT (p.Phe1863Serfs*8)	c.6037C>T (p.Arg2013*)	Current
ODA28	*DNAH5*	c.8383C>T (p.Arg2795*)	c.8383C>T (p.Arg2795*)	Current
ODA29	*DNAH5*	c.10815delT (p.Pro3606Hisfs*23)	c.3037_3040delAGCG (p.Val1014Leufs*20)	9
ODA30	*DNAH5*	c.10815delT (p.Pro3606Hisfs*23)	c.3037_3040delAGCG (p.Val1014Leufs*20)	Current
ODA31	*DNAH5*	c.13486C>T (p.Arg4496*)	4.4-kb duplication including exons 41-42 (p.?)	3
ODA32	*DNAH5*	c.9286C>T (p.Arg3096*)	c.6086delG (p.GLy2029Valfs*25)	9

ODA33	*DNAH5*	c.4237C>T (p.Gln1413*)	1.6-kb deletion including exon 2 (p.?)	3
ODA34	*DNAH5*	c.10815delT (p.Pro3606Hisfs*23)	c.3598+2T>C (p.?)	Current
ODA35	*DNAH5*	c.10815delT (p.Pro3606Hisfs*23)	c.8010+3A>G (p.?)	Current
ODA36	*DNAH5*	c.12617G>A (p.Trp4206*)	c.12617G>A (p.Trp4206*)	Current
ODA37	*DNAH5*	c.13486C>T (p.Arg4496*)	c.8092_8097delGTGGAC (p.Val2698_Asp2699del)[§]	Current
ODA38	*DNAH5*	c.832delG (p.Ala278Argfs*27)	c.10815delT (p.Pro3606Hisfs*23)	Current
ODA39	*DNAH5*	c.13458dupT (p.Asn4487*)	c.11308A>G (p.Ser3770Gly)[§]	9
ODA40	*DNAI1*	c.1212T>G (p.Tyr404*)	c.1644G>A (p.Trp548*)	6
ODA41	*DNAI1*	c.48+2dupT (p.splice)	c.1703G>C (p.Trp568Ser)	10
ODA42	*DNAI1*	c.48+2dupT (p.splice)	c.1703G>C (p.Trp568Ser)	10
ODA43	*DNAI1*	c.1212T>G (p.Tyr404*)	c.1222G>A (p.Val408Met)	11
ODA44	*DNAI1*	c.1462delC (p.Pro488Leufs*25)	c.1612G>A (p.Ala538Thr)	Current
ODA45	*DNAI1*	c.48+2dupT (p.splice)	c.1612G>A (p.Ala538Thr)	Current
ODA46	*DNAI1*	c.48+2dupT (p.splice)	c.48+2dupT (p.splice)	Current
ODA47	*DNAI2*	c.1304G>A (p.Trp435*)	c.1304G>A (p.Trp435*)	2
ODA48	*DNAI2*	c.1304G>A (p.Trp435*)	c.1304G>A (p.Trp435*)	2
ODA49	*DNAI2*	c.739C>T (p.Arg247*)	c.739C>T (p.Arg247*)	9
ODA50	*DNAI2*	c.1304G>A (p.Trp435*)	c.1357dupG (p.Glu453Glyfs*40)	7
ODA51	*DNAI2*	c.1304G>A (p.Trp435*)	c.1357dupG (p.Glu453Glyfs*40)	7
ODA52	Panel negative	.	.	
ODA53	Panel negative	.	.	
ODA54	Panel negative	.	.	
ODA55	Panel negative	.	.	
		ODA/IDA Group (n=20)		
O+IDA01	*CCDC103*	c.144-1G>A (p.?)	c.461A>C (p.His154Pro)	Current
O+IDA02[#]	*CCDC103*	c.461A>C (p.His154Pro)	c.461A>C (p.His154Pro)	7, 9
O+IDA03	*DNAAF1/LRRC50*	c.831C>G (p.Tyr277*)	c.831C>G (p.Tyr277*)	Current
O+IDA04	*DNAAF1/LRRC50*	deletion including exon 5 (p.?)	c.778C>T (p.Gln260*)	Current
O+IDA05	*DNAAF2/KTU*	c.31delG (p.Glu11Argfs*4)	c.31delG (p.Glu11Argfs*4)	9
O+IDA06	*DNAAF3*	c.378delG (p.His127Thrfs*34)	deletion including exons 7-12 (p.?)	Current
O+IDA07	*DYX1C1*	3549-bp deletion including exon 7 (p.?)	c.988C>T (p.Arg330Trp)[§]	3
O+IDA08	*DYX1C1*	3549-bp deletion including exon 7 (p.?)	c.325G>T (p.Glu109*)	Current
O+IDA09	*DYX1C1*	3549-bp deletion including exon 7 (p.?)	c.325G>T (p.Glu109*)	Current
O+IDA10	*HEATR2/DNAAF5*	c.2384T>C (p.Leu795Pro)	c.2384T>C (p.Leu795Pro)	8, 12
O+IDA11	*HEATR2/DNAAF5*	c.2384T>C (p.Leu795Pro)	c.2384T>C (p.Leu795Pro)	8, 12
O+IDA12	*LRRC6*	c.630delG (p.Trp210Cysfs*12)	c.630delG (p.Trp210Cysfs*12)	7
O+IDA13	*LRRC6*	c.630delG (p.Trp210Cysfs*12)	c.630delG (p.Trp210Cysfs*12)	7
O+IDA14	*LRRC6*	c.630delG (p.Trp210Cysfs*12)	c.630delG (p.Trp210Cysfs*12)	7
O+IDA15	*PIH1D3*	c.332+1G>A (p.?)	none - x-linked in male	Current
O+IDA16	*SPAG1*	c.2014C>T (p.Gln672*)	c.2014C>T (p.Gln672*)	13
O+IDA17	*SPAG1*	c.2014C>T (p.Gln672*)	c.1119delC (p.Ala374Argfs*16)	Current

O+IDA18	Panel negative	.	.	
O+IDA19	Panel negative	.	.	
O+IDA20	Panel negative	.	.	
		IDA+MTD Group (n=41)		
I+M01[#]	*CCDC39*	c.1167+1261A>G (p.Glu390Serfs*5)	c.1356C>A (p.Tyr452*)	Current
I+M02	*CCDC39*	c.830_831delCA (p.Thr277Argfs*3)	c.830_831delCA (p.Thr277Argfs*3)	14
I+M03	*CCDC39*	c.1665+1G>A (p.?)	c.350A>G (p.Asp117Gly)[§]	Current
I+M04	*CCDC39*	c.610-2A>G (p.?)	c.830_831delCA (p.Thr277Argfs*3)	Current
I+M05	*CCDC39*	c.436delT (p.Trp146Glyfs*2)	c.830_831delCA (p.Thr277Argfs*3)	Current
I+M06	*CCDC39*	c.1045delA (p.Thr349Leufs*7)	c.1045delA (p.Thr349Leufs*7)	Current
I+M07	*CCDC39*	c.830_831delCA (p.Thr277Argfs*3)	c.1795C>T (p.Arg599*)	9
I+M08	*CCDC39*	c.1789G>T (p.Glu597*)	c.2497_98delCA (p.Gln833Valfs*6)	7
I+M09	*CCDC39*	c.1789G>T (p.Glu597*)	c.2497_98delCA (p.Gln833Valfs*6)	7
I+M10	*CCDC39*	c.1167+1261A>G (p.Glu390Serfs*5)	c.1167+1261A>G (p.Glu390Serfs*5)	Current
I+M11	*CCDC39*	c.610-2A>G (p.?)	c.1795C>T (p.Arg599*)	Current
I+M12	*CCDC39*	c.357+1G>C (p.?)	c.2190delA (p.Glu731Asnfs*31)	Current
I+M13	*CCDC39*	c.1356C>A (p.Tyr452*)	c.1665+1G>A (p.?)	Current
I+M14	*CCDC39*	c.2201_2202delGA (p.Arg734Serfs*3)	c.2201_2202delGA (p.Arg734Serfs*3)	Current
I+M15	*CCDC39*	c.830_831delCA (p.Thr277Argfs*3)	c.830_831delCA (p.Thr277Argfs*3)	9
I+M16	*CCDC39*	c.1484_1485delAA (p.Lys495Ilefs*15)	c.1484_1485delAA (p.Lys495Ilefs*15)	Current
I+M17	*CCDC40*	c.248delC (p.Ala83Valfs*84)	c.248delC (p.Ala83Valfs*84)	14
I+M18	*CCDC40*	c.1093G>T (p.Glu365*)	c.3358C>T (p.Gln1120*)	Current
I+M19	*CCDC40*	c.1093G>T (p.Glu365*)	c.3358C>T (p.Gln1120*)	Current
I+M20	*CCDC40*	c.1093G>T (p.Glu365*)	c.3358C>T (p.Gln1120*)	Current
I+M21	*CCDC40*	c.248delC (p.Ala83Valfs*84)	gene deletion (p.0)	14, Current
I+M22[#]	*CCDC40*	c.248delC (p.Ala83Valfs*84)	c.248delC (p.Ala83Valfs*84)	7
I+M23	*CCDC40*	c.93+2T>C (p.?)	c.248delC (p.Ala83Valfs*84)	Current
I+M24	*CCDC40*	c.248delC (p.Ala83Valfs*84)	c.961C>T (p.Arg321*)	14
I+M25	*CCDC40*	c.1416delG (p.Ile473Phefs*2)	c.1416delG (p.Ile473Phefs*2)	7, 9
I+M26	*CCDC40*	c.1416delG (p.Ile473Phefs*2)	c.1416delG (p.Ile473Phefs*2)	7, 9
I+M27	*CCDC40*	c.248delC (p.Ala83Valfs*84)	c.1312A>T (p.Lys438*)	15
I+M28	*CCDC40*	c.961C>T (p.Arg321*)	c.3129delC (p.Phe1044Serfs*35)	14
I+M29	*CCDC40*	c.961C>T (p.Arg321*)	c.3129delC (p.Phe1044Serfs*35)	14
I+M30	*CCDC40*	c.248delC (p.Ala83Valfs*84)	c.248delC (p.Ala83Valfs*84)	14
I+M31	*CCDC40*	c.248delC (p.Ala83Valfs*84)	c.248delC (p.Ala83Valfs*84)	7
I+M32	*CCDC40*	c.248delC (p.Ala83Valfs*84)	c.248delC (p.Ala83Valfs*84)	14
I+M33	*CCDC40*	c.1345C>T (p.Arg449*)	c.2712-1G>T (p.?)	14
I+M34	*CCDC40*	c.248delC (p.Ala83Valfs*84)	c.248delC (p.Ala83Valfs*84)	14
I+M35	Panel negative	.	.	
I+M36	Panel negative	.	.	
I+M37	Panel negative	.	.	

I+M38	Panel negative	.	.	
I+M39	Panel negative	.	.	
I+M40	Panel negative	.	.	
I+M41	Panel negative	.	.	
Other Group (n=21)				
other01[#]	*CCNO*	c.248_252dupTGCCC (p.Gly85Cysfs*11)	c.248_252dupTGCCC (p.Gly85Cysfs*11)	Current
other02[#]	*CCNO*	c.248_252dupTGCCC (p.Gly85Cysfs*11)	c.258_262dupGGCCC (p.Gln88Argfs*8)	Current
other03[#]	*CCNO*	c.248_252dupTGCCC (p.Gly85Cysfs*11)	c.258_262dupGGCCC (p.Gln88Argfs*8)	Current
other04[#]	*CCNO*	c.248_252dupTGCCC (p.Gly85Cysfs*11)	c.248_252dupTGCCC (p.Gly85Cysfs*11)	Current
other05	*DNAH11*	c.2491C>T (p.Gln831*)	c.3871G>A (p.Ala1291Thr)[§]	7
other06	*DNAH11*	c.2491C>T (p.Gln831*)	c.3871G>A (p.Ala1291Thr)[§]	7
other07	*DNAH11*	c.6244C>T (p.Arg2082*)	c.11929G>T (p.Glu3977*)	16
other08	*DNAH11*	c.13065_13067delCCT (p.Leu4356del)[§]	c.13075C>T (p.Arg4359*)	16
other09	*DNAH11*	c.4438C>T (p.Arg1480*)	c.8698C>T (p.Arg2900*)	7
other10	*DNAH11*	c.4438C>T (p.Arg1480*)	c.8698C>T (p.Arg2900*)	7
other11	*DNAH11*	c.10285C>A (p.Arg3429Ser)[§]	32.29-kb duplication including exons 7-14 (p.?)	3,9
other12	*DNAH11*	c.5778+1G>A (p.Val1821Thrfs*8)	c.13061T>A (p.Leu4354His)[§]	16
other13	*DNAH11*	c.3901G>T (p.Glu1301*)	c.11804C>T (p.Pro3935Leu)[§]	16
other14	*DNAH11*	c.6130C>T (p.Arg2044*)	deletion including exons 68-74 (p.?)	Current
other15	*DNAH11*	c.6244C>T (p.Arg2082*)	duplication including exons 41-42	Current
other16	*RPGR*	c.1059delG; c.1059+1_1059+2delGT (p.?)	none - x-linked in male	Current
other17	*RSPH1*	c.275-2A>C (p.Gly92Alafs*10)	c.275-2A>C (p.Gly92Alafs*10)	17
other18	*RSPH4A*	c.921+3_6delAAGT (p.Tyr230Glnfs*8)	c.1732_1733delGA (p.Asp578Argfs*3)	18
other19	*RSPH4A*	c.116C>A (p.Ser39*)	c.1662+2_5delTAGG (p.?)	18
other20	*RSPH9*	c.244delT (p.Trp82Glyfs*18)	c.244delT (p.Trp82Glyfs*18)	Current
other21	Panel negative	.	.	

[#] A total of 7 participants had inadequate EM. The remaining 130 participants had EM findings that were congruent with ultrastructural defects predicted by genetics.

[§] A total of 10 private alleles (either missense or in-frame alleles), that we considered as probably contributing to the disease phenotype based on criteria outlined in methods. Note: Based on the current ACMG criteria for variant classification (19), private missense or in-frame variants without functional studies are considered variants of uncertain significance (VUS).

Sensitivity Analysis Removing Preschool Spirometry: Because there is limited information regarding early lung function in PCD, we included preschool spirometry for our main analysis. However, preschool spirometry may not equate to spirometry in older children and adults; therefore, we conducted a sensitivity analysis of our data with and without preschool spirometry. We defined the preschool age range as children between the ages of 3 and 5 years of age. There were 42 measurements performed between the ages of 3 and 5 years. Of these 42 measurements, 4 were conducted at 3 years of age, 14 were conducted at 4 years of age and 24 at 5 years of age.

When we removed the measurements for the 3 to 5 year old group and compared the ODA group to the IDA/CA/MTD group, the IDA/CA/MTD group continued to have significantly lower percent predicted FEV_1 values compared to the ODA group (p= 0.0002). Similarly for the genotype grouping with removal of the 3 to 5 year old group, *CCDC39* and *CCDC40* had significantly lower FEV_1 values compared to the *DNAH5* group (p = 0.001). When we removed the spirometry measurements for the children that were 3 to 5 years of age, the decline in the slope for FEV_1 remained significant for the IDA/CA/MTD group (p=0.01); furthermore, the decline in the slope for FEV_1 for the ODA group became significant with a p value of 0.04.

References

1. Hjeij R, Lindstrand A, Francis R, Zariwala MA, Liu X, Li Y, Damerla R, Dougherty GW, Abouhamed M, Olbrich H, Loges NT, Pennekamp P, Davis EE, Carvalho CM, Pehlivan D, Werner C, Raidt J, Köhler G, Häffner K, Reyes-Mugica M, Lupski JR, Leigh MW, Rosenfeld M, Morgan LC, Knowles MR, Lo CW, Katsanis N, Omran H. ARMC4 mutations cause primary ciliary dyskinesia with randomization of left/right body asymmetry. *Am J Hum Genet* 2013; 93:357-67.
2. Knowles MR, Leigh MW, Ostrowski LE, Huang L, Carson JL, Hazucha MJ, Yin W, Berg JS, Davis SD, Dell SD, Ferkol TW, Rosenfeld M, Sagel SD, Milla CE, Olivier KN, Turner EH, Lewis AP, Bamshad MJ, Nickerson DA, Shendure J, Zariwala MA. Exome sequencing identifies mutations in CCDC114 as a cause of primary ciliary dyskinesia. *Am J Hum Genet* 2013; 92:99-106.
3. Marshall CR, Scherer SW, Zariwala MA, Lau L, Paton TA, Stockley T, Jobling RK, Ray PN, Knowles MR; FORGE Canada Consortium, Hall DA, Dell SD, Kim RH. Whole-Exome Sequencing and Targeted Copy Number Analysis in Primary Ciliary Dyskinesia. *G3* (Bethesda). 2015; 5:1775-81.
4. Hornef N, Olbrich H, Horvath J, Zariwala MA, Fliegauf M, Loges NT, Wildhaber J, Noone PG, Kennedy M, Antonarakis SE, Blouin JL, Bartoloni L, Nüsslein T, Ahrens P, Griese M, Kuhl H, Sudbrak R, Knowles MR, Reinhardt R, Omran H. DNAH5 mutations are a common cause of primary ciliary dyskinesia with outer dynein arm defects. *Am J Respir Crit Care Med* 2006; 174:120-6.

5. Olbrich H, Häffner K, Kispert A, Völkel A, Volz A, Sasmaz G, Reinhardt R, Hennig S, Lehrach H, Konietzko N, Zariwala M, Noone PG, Knowles M, Mitchison HM, Meeks M, Chung EM, Hildebrandt F, Sudbrak R, Omran H. Mutations in DNAH5 cause primary ciliary dyskinesia and randomization of left-right asymmetry. *Nat Genet* 2002; 30:143-4.

6. Berg JS, Evans JP, Leigh MW, Omran H, Bizon C, Mane K, Knowles MR, Weck KE, Zariwala MA. Next generation massively parallel sequencing of targeted exomes to identify genetic mutations in primary ciliary dyskinesia: implications for application to clinical testing. *Genet Med* 2011; 13:218-29.

7. Zariwala MA, Gee HY, Kurkowiak M, Al-Mutairi DA, Leigh MW, Hurd TW, Hjeij R, Dell SD, Chaki M, Dougherty GW, Adan M, Spear PC, Esteve-Rudd J, Loges NT, Rosenfeld M, Diaz KA, Olbrich H, Wolf WE, Sheridan E, Batten TF, Halbritter J, Porath JD, Kohl S, Lovric S, Hwang DY, Pittman JE, Burns KA, Ferkol TW, Sagel SD, Olivier KN, Morgan LC, Werner C, Raidt J, Pennekamp P, Sun Z, Zhou W, Airik R, Natarajan S, Allen SJ, Amirav I, Wieczorek D, Landwehr K, Nielsen K, Schwerk N, Sertic J, Köhler G, Washburn J, Levy S, Fan S, Koerner-Rettberg C, Amselem S, Williams DS, Mitchell BJ, Drummond IA, Otto EA, Omran H, Knowles MR, Hildebrandt F. ZMYND10 is mutated in primary ciliary dyskinesia and interacts with LRRC6. *Am J Hum Genet* 2013; 93:336-45.

8. Ferkol TW, Puffenberger EG, Lie H, Helms C, Strauss KA, Bowcock A, Carson JL, Hazucha M, Morton DH, Patel AC, Leigh MW, Knowles MR, Zariwala MA. Primary ciliary dyskinesia-causing mutations in Amish and Mennonite communities. *J Pediatr* 2013; 163:383-7.

9. Kim RH, A Hall D, Cutz E, Knowles MR, Nelligan KA, Nykamp K, Zariwala MA, Dell SD. The role of molecular genetic analysis in the diagnosis of primary ciliary dyskinesia. *Ann Am Thorac Soc* 2014; 11:351-9.

10. Zariwala M, Noone PG, Sannuti A, Minnix S, Zhou Z, Leigh MW, Hazucha M, Carson JL, Knowles MR. Germline mutations in an intermediate chain dynein cause primary ciliary dyskinesia. *Am J Respir Cell Mol Biol* 2001; 25:577-83.

11. Zariwala MA, Leigh MW, Ceppa F, Kennedy MP, Noone PG, Carson JL, Hazucha MJ, Lori A, Horvath J, Olbrich H, Loges NT, Bridoux AM, Pennarun G, Duriez B, Escudier E, Mitchison HM, Chodhari R, Chung EM, Morgan LC, de Iongh RU, Rutland J, Pradal U, Omran H, Amselem S, Knowles MR. Mutations of DNAI1 in primary ciliary dyskinesia: evidence of founder effect in a common mutation. *Am J Respir Crit Care Med* 2006; 174:858-66.

12. Horani A, Druley TE, Zariwala MA, Patel AC, Levinson BT, Van Arendonk LG, Thornton KC, Giacalone JC, Albee AJ, Wilson KS, Turner EH, Nickerson DA, Shendure J, Bayly PV, Leigh MW, Knowles MR, Brody SL, Dutcher SK, Ferkol TW. Whole-exome capture and sequencing identifies HEATR2 mutation as a cause of primary ciliary dyskinesia. *Am J Hum Genet* 2012; 91:685-93.

13. Knowles MR, Ostrowski LE, Loges NT, Hurd T, Leigh MW, Huang L, Wolf WE, Carson JL, Hazucha MJ, Yin W, Davis SD, Dell SD, Ferkol TW, Sagel SD, Olivier KN, Jahnke C, Olbrich H, Werner C, Raidt J, Wallmeier J, Pennekamp P, Dougherty GW, Hjeij R, Gee HY, Otto EA, Halbritter J, Chaki M, Diaz KA, Braun DA, Porath JD, Schueler M, Baktai G, Griese M, Turner EH, Lewis AP, Bamshad MJ, Nickerson DA, Hildebrandt F, Shendure J, Omran H,

Zariwala MA. Mutations in SPAG1 cause primary ciliary dyskinesia associated with defective outer and inner dynein arms. *Am J Hum Genet* 2013; 93:711-20.

14. Antony D, Becker-Heck A, Zariwala MA, Schmidts M, Onoufriadis A, Forouhan M, Wilson R, Taylor-Cox T, Dewar A, Jackson C, Goggin P, Loges NT, Olbrich H, Jaspers M, Jorissen M, Leigh MW, Wolf WE, Daniels ML, Noone PG, Ferkol TW, Sagel SD, Rosenfeld M, Rutman A, Dixit A, O'Callaghan C, Lucas JS, Hogg C, Scambler PJ, Emes RD; Uk10k, Chung EM, Shoemark A, Knowles MR, Omran H, Mitchison HM.Mutations in CCDC39 and CCDC40 are the major cause of primary ciliary dyskinesia with axonemal disorganization and absent inner dynein arms. *Hum Mutat* 2013; 34:462-72.

15. Nakhleh N, Francis R, Giese RA, Tian X, Li Y, Zariwala MA, Yagi H, Khalifa O, Kureshi S, Chatterjee B, Sabol SL, Swisher M, Connelly PS, Daniels MP, Srinivasan A, Kuehl K, Kravitz N, Burns K, Sami I, Omran H, Barmada M, Olivier K, Chawla KK, Leigh M, Jonas R, Knowles M, Leatherbury L, Lo CW. High prevalence of respiratory ciliary dysfunction in congenital heart disease patients with heterotaxy. *Circulation* 2012; 125:2232-42.

16. Knowles MR, Leigh MW, Carson JL, Davis SD, Dell SD, Ferkol TW, Olivier KN, Sagel SD, Rosenfeld M, Burns KA, Minnix SL, Armstrong MC, Lori A, Hazucha MJ, Loges NT, Olbrich H, Becker-Heck A, Schmidts M, Werner C, Omran H, Zariwala MA. Mutations of DNAH11 in patients with primary ciliary dyskinesia with normal ciliary ultrastructure. *Thorax* 2012; 67:433-41.

17. Knowles MR, Ostrowski LE, Leigh MW, Sears PR, Davis SD, Wolf WE, Hazucha MJ, Carson JL, Olivier KN, Sagel SD, Rosenfeld M, Ferkol TW, Dell SD, Milla CE, Randell SH, Yin W, Sannuti A, Metjian HM, Noone PG, Noone PJ, Olson CA, Patrone MV, Dang H, Lee HS,

Hurd TW, Gee HY, Otto EA, Halbritter J, Kohl S, Kircher M, Krischer J, Bamshad MJ, Nickerson DA, Hildebrandt F, Shendure J, Zariwala MA. Mutations in RSPH1 cause primary ciliary dyskinesia with a unique clinical and ciliary phenotype. *Am J Respir Crit Care Med* 2014; 189:707-17.

18. Daniels ML, Leigh MW, Davis SD, Armstrong MC, Carson JL, Hazucha M, Dell SD, Eriksson M, Collins FS, Knowles MR, Zariwala MA. Founder mutation in RSPH4A identified in patients of Hispanic descent with primary ciliary dyskinesia. *Hum Mutat* 2013; 34:1352-6.

19. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17:405-24.