

Laterality Defects Other Than Situs Inversus Totalis in Primary Ciliary Dyskinesia

Insights Into Situs Ambiguus and Heterotaxy

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BACKGROUND: Motile cilia dysfunction causes primary ciliary dyskinesia (PCD), situs inversus totalis (SI), and a spectrum of laterality defects, yet the prevalence of laterality defects other than SI in PCD has not been prospectively studied.

METHODS: In this prospective study, participants with suspected PCD were referred to our multisite consortium. We measured nasal nitric oxide (nNO) level, examined cilia with electron microscopy, and analyzed PCD-causing gene mutations. Situs was classified as (1) situs solitus (SS), (2) SI, or (3) situs ambiguus (SA), including heterotaxy. Participants with hallmark electron microscopic defects, biallelic gene mutations, or both were considered to have classic PCD.

RESULTS: Of 767 participants (median age, 8.1 years, range, 0.1-58 years), classic PCD was defined in 305, including 143 (46.9%), 125 (41.0%), and 37 (12.1%) with SS, SI, and SA, respectively. A spectrum of laterality defects was identified with classic PCD, including 2.6% and 2.3% with SA plus complex or simple cardiac defects, respectively; 4.6% with SA but no cardiac defect; and 2.6% with an isolated possible laterality defect. Participants with SA and classic PCD had a higher prevalence of PCD-associated respiratory symptoms vs SA control participants (year-round wet cough, $P < .001$; year-round nasal congestion, $P = .015$; neonatal respiratory distress, $P = .009$; digital clubbing, $P = .021$) and lower nNO levels (median, 12 nL/min vs 252 nL/min; $P < .001$).

CONCLUSIONS: At least 12.1% of patients with classic PCD have SA and laterality defects ranging from classic heterotaxy to subtle laterality defects. Specific clinical features of PCD and low nNO levels help to identify PCD in patients with laterality defects.

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ABBREVIATIONS: CA = central apparatus; d-TGA = dextro-transposition of the great arteries; EM = electron microscopic; IDA = inner dynein arm; nNO = nasal nitric oxide; ODA = outer dynein arm; PCD = primary ciliary dyskinesia; SA = situs ambiguus; SI = situs inversus totalis; SS = situs solitus

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Primary ciliary dyskinesia (PCD) is a rare autosomal recessive disease with an estimated prevalence of one in 16,000.^{1,2} Patients with PCD display neonatal respiratory distress, chronic sinopulmonary disease, bronchiectasis, recurrent otitis media, and infertility.³ Situs inversus totalis (SI), or mirror-image organ arrangement, occurs in slightly < 50% of patients with PCD; however, a subset of patients with PCD have organ laterality defects that do not meet a classic definition of SI. Instead, findings in this subset of patients correspond to an intermediate classification defined as situs ambiguus (SA) with a spectrum of organ laterality defects. Classification of these defects is complicated because different specialists tend to use different definitions for SA and heterotaxy.⁴⁻⁸ Some clinicians interchange the terms “situs ambiguus” and “heterotaxy”; however, cardiologists define heterotaxy as a subset of SA with specific congenital heart defects (eg, common atrium, levo-transposition of great arteries). Geneticists have expanded the cardiology definition to include combinations of abdominal defects (eg, asplenia, polysplenia) or vascular defects (eg, interrupted inferior vena cava). Despite this confusion, previous studies of heterotaxy reported a prevalence of one in 10,000, with multimodal forms of inheritance.^{9,10} We define in this article any laterality defect other than SI as

SA and only those with SA combined with complex heart lesions as heterotaxy.

Studies have demonstrated that cilia dysfunction in embryonic nodal plate cells results in laterality defects and congenital heart disease.^{11,12} In addition, a link between embryonic cilia dysfunction and respiratory cilia dysfunction in mice demonstrates that mutations in *Dnai2*

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and *Dnahc5* (homologs to *DNAI2* and *DNAH5* in humans) lead to heterotaxy and PCD with outer dynein arm (ODA) defects.^{13,14} Kennedy et al¹⁵ demonstrated retrospectively that SA anomalies and heterotaxy are present in at least 6.3% of an international PCD population. Most patients with SA, particularly those with congenital heart disease, have complicated medical courses, including neonatal respiratory distress, cyanosis, and pneumonia. These respiratory manifestations are commonly attributed to a cardiac origin without consideration of possible respiratory cilia dysfunction.^{16,17} The present prospective study examined the prevalence of laterality defects other than SI in a PCD population and determined which clinical and diagnostic characteristics suggest PCD in a population with complex situs issues.

Materials and Methods

Through our multicenter Genetic Diseases of Mucociliary Clearance Consortium, symptomatic adults and children were referred for previously diagnosed or suspected PCD. Medical records were reviewed and evaluations conducted at one of seven sites in California, Colorado, Maryland, Missouri, North Carolina, Washington, and Ontario, Canada, between May 2006 and September 2012.

Initial situs status was determined by physicians at local consortium sites through review of radiology, surgery, and cardiology reports and radiology images from participant medical records. Additionally, standardized questions to detect situs anomalies were asked of each participant. One consortium physician (A. J. S.) reviewed each potential SA case in a nonblinded fashion, verifying the situs lesions through source documentation before assigning the ultimate situs designation. Participants were grouped into one of the following situs categories: (1) situs

solitus (SS); (2) SI; or (3) SA, which includes heterotaxy (Fig 1). Next, we used the congenital heart disease classification schema developed by Botto et al¹⁸ to assign participants with SA to one of the following SA subgroups: (1) SA with complex cardiovascular malformation (heterotaxy to most cardiologists), including cardiac isomerism or any cardiovascular defect or groups of defects that do not fit into standard cardiac phenotype classification or associations; (2) SA with simple cardiovascular malformation, including cardiovascular malformations within standard cardiac phenotype classification or associations; (3) SA without cardiac malformation, including vascular, abdominal, or laterality defects but normal cardiac status; and (4) isolated possible laterality defect consisting of one solitary defect, possibly related to issues of laterality, in either cardiac or noncardiac systems. Laterality defects included in each group are detailed in Table 1.

Nasal nitric oxide (nNO) level was measured in participants with either a CLD 88 series (Eco Medics AG), a NIOX Flex (Aerocrine), or a Sievers

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Dr Shapiro was affiliated with the University of North Carolina when this research began.

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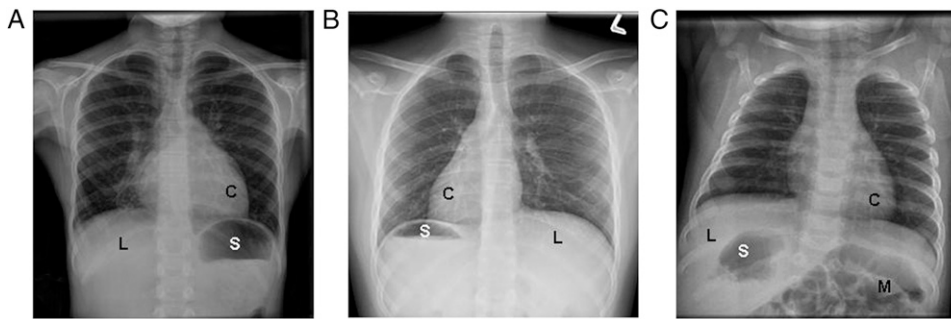


Figure 1 – Examples of laterality defects on radiology imaging in various situs groups in the study population. A, A participant with situs solitus, or normal organ arrangement, with left cardiac apex, left-sided stomach bubble, and right-sided liver. B, A patient with situs inversus totalis, or mirror-image organ arrangement, with right-sided cardiac apex, right-sided stomach bubble, and left-sided liver. C, A patient with situs ambiguus with left-sided cardiac apex, right-sided stomach bubble, right-sided liver, and intestinal malrotation who also has right-sided polysplenia visualized on CT scan. C = cardiac apex; L = liver; M = intestinal malrotation; S = stomach.

NOA 280i (General Electric Company) chemiluminescence analyzer, using standard operating procedures. In participants who could cooperate with standard velum closure maneuvers (generally aged > 5 years), values were obtained during a 5-s plateau while exhaling through a resistor.^{19,20} Reported values are the mean of three maneuvers each in the left- and right-side naris. Otherwise, nNO values were obtained during tidal breathing, using the mean of the five highest tidal peaks from each naris.²¹ Measured nNO concentration was multiplied by sampling flow to determine nNO production in nanoliters per minute. For velum closure nNO and tidal breathing nNO, values < 77 nL/min^{20,22,23} and < 40 nL/min,^{21,24} respectively, were considered low and within PCD range. Velum closure nNO measurements were also collected from 77 healthy pediatric control participants.

Nasal ciliary biopsy specimens were processed for ultrastructural analysis through transmission electron microscopy (Zeiss EM900; Carl Zeiss AG). Photomicrographs were reviewed in a blinded fashion by

three independent observers. Each reviewer separately determined whether hallmark disease-causing defects were present in the ODAs, inner dynein arms (IDAs), or central apparatus (CA).^{3,25,26} Genetic testing for known mutations in PCD-causing genes was performed in cases with hallmark electron microscopic (EM) defects and strong clinical phenotypes. We tested *DNAI1* and *DNAH5* for patients with ODA defects; *DNAAF2*, *DYX1C1*, *HEATR2*, and *LRR6* for patients with ODA plus IDA defects; *CCDC39* and *CCDC40* for patients with IDA plus CA and microtubule disorganization defects; *DNAH11* for patients with normal ciliary ultrastructure; and all of these genes in patients where ciliary ultrastructure was not available. Both EM and genetic testing were performed in centralized laboratories at the University of North Carolina using standard operating procedures.

Participants with biallelic mutations in PCD-causing genes, hallmark EM defects, or both were given a diagnosis of classic PCD. All remaining participants were labeled as without classic PCD and grouped

TABLE 1] Laterality Defects Included in SA Subgroups

SA + Complex CVM (Heterotaxy)	SA + Simple CVM	SA Without Cardiac	Isolated Possible Laterality Defect
<p>SA plus any of the following cardiac issues:</p> <ul style="list-style-type: none"> • Cardiac isomerism • Hypoplastic ventricle with other cardiac lesions (except ASD or APVR alone) • I-TGA + LVOTO (PS) • Any defects that do not fit into a standard cardiac phenotype classification or standard associations 	<p>SA plus any of the following standard cardiac phenotypes or associations:</p> <ul style="list-style-type: none"> • Dextrocardia • Mesocardia • ASD • VSD • AVSD • Common atrium • Tetralogy of Fallot • Pulmonary stenosis or atresia • Overriding aorta • DORV • Ebstein anomaly • Tricuspid atresia • I-TGA • Truncus defect • HLHS + VSD/APVR 	<p>SA including any of the following defects:</p> <p>Vascular</p> <ul style="list-style-type: none"> • APVR • Right aortic arch • Bilateral or left superior vena cava • Interrupted or duplicated inferior vena cava <p>Abdomen</p> <ul style="list-style-type: none"> • Asplenia or polysplenia • Intestinal malrotation • Midline liver • Left-sided liver • Right-sided spleen or stomach <p>Pulmonary</p> <ul style="list-style-type: none"> • Left- or right-side pulmonary isomerism 	<p>Any solitary lesion that may be associated with laterality defects, including cardiac or noncardiac lesions</p>

Classification criteria used from Botto et al.¹⁸ APVR = anomalous pulmonary venous return; ASD = atrial septal defect; AVSD = atrioventricular septal defect; CVM = cardiovascular malformation; DORV = double-outlet right ventricle; HLHS = hypoplastic left heart syndrome; I-TGA = levo-transposition of the great arteries; LVOTO = left ventricular outflow tract obstruction; PS = pulmonary stenosis; SA = situs ambiguus; VSD = ventricular septal defect.

according to normal or low nNO levels. Those with normal nNO levels above our cutoff formed the SA control group. Final data analyses were limited to participants aged > 1 year in whom nNO measurements were more reliable and adequate clinical symptom history had been established. Continuous data and categorical data were analyzed using Wilcoxon rank sum and χ^2 or Fisher exact tests, respec-

tively. Multiple group comparisons were performed using Kruskal-Wallis test. Statistical significance was defined as $P < .05$ for two-way comparisons and as $P \leq .03$ with Bonferroni correction for post hoc comparisons of multiple groups against classic PCD. Institutional review boards at the participating institutions approved this protocol (e-Appendix 1).

Results

Overall, 305 of 767 participants (39.8%) were given a diagnosis of classic PCD, including 37 (12.1%) with SA and classic PCD. This group with SA and classic PCD included eight with SA plus complex cardiovascular malformation, seven with SA plus simple cardiovascular malformation, 14 with SA without cardiac malformation, and eight with a possible isolated laterality defect (Fig 2, e-Table 1). SA was identified in 41 of 462 participants without classic PCD. Twenty-five of these participants had normal nNO levels above our cutoff and formed the SA control group. Nine participants with SA without classic PCD had low nNO levels under our cutoff, and seven others were not included in the final data analysis because they were aged < 1 year. The median age of participants with SA and classic PCD was significantly greater than that of participants in the SA control group (Table 2). No specific organ laterality defects were more prevalent in classic PCD; however, dextro-transposition of the great arteries (d-TGA) was more prevalent in SA without classic PCD than in classic PCD. Asplenia and atrioventricular septal defect were more prevalent in SA without classic PCD and nNO levels below our cutoff than in classic PCD (Table 2). Nine participants with SA and classic PCD also had siblings enrolled in the protocol, but none of the siblings had SA.

Nineteen of the 37 participants (51%) with SA and classic PCD had ODA defects alone on electron micros-

copy; eight (22%) had ODA plus IDA defects; five (13%) had IDA plus CA and microtubule disorganization defects; four (11%) had inadequate or indeterminate (no hallmark PCD-causing defects, but not normal either) samples; one (3%) had normal EM findings; and none had isolated CA, IDA, or radial spoke defects. Distribution of ODA defects, ODA plus IDA defects, and IDA plus CA and microtubule disorganization defects in SA was similar to that in SS and SI. None of the participants who had radial spoke defects had laterality defects. There were no significant correlations between genetic mutation and situs anomalies. Distribution of EM defects within the SA population did not correspond to specific laterality defects (Table 3).

Year-round wet cough, year-round nasal congestion, neonatal respiratory distress, and digital clubbing were significantly more prevalent in participants with classic PCD than in SA control participants (Table 4). The prevalence of digital clubbing did not correlate with participant age (median age with clubbing, 14 years; median age with no clubbing, 8.5 years; $P = .19$). The prevalence of neonatal respiratory distress in classic PCD was increased despite a higher prevalence of congenital heart disease, which is known to cause respiratory distress, in SA control participants. The age of onset for nasal congestion was significantly earlier in classic PCD, but age of onset for other sino-oto-pulmonary symptoms was similar in both groups and mainly within the first year of life (Table 4).

Levels of nNO measured during velum closure were significantly lower in participants with classic PCD (median, 12 nL/min; range, 4-48 nL/min; $n = 30$) than in all participants without classic PCD (median nNO level above cutoff, 252 nL/min [range, 137-338 nL/min; $n = 11$]; median nNO level below cutoff, 29 nL/min [range 8-66, $n = 8$]; $P < .001$). Levels of nNO during tidal breathing were also significantly lower in participants with classic PCD (median, 8.5 nL/min; range, 5-16 nL/min; $n = 4$) than in those without classic PCD (median, 128 nL/min; range, 37-276; $n = 14$; $P = .003$). For the 77 healthy control participants, median velum closure nNO level was 286 nL/min, which was significantly higher than in participants with classic PCD

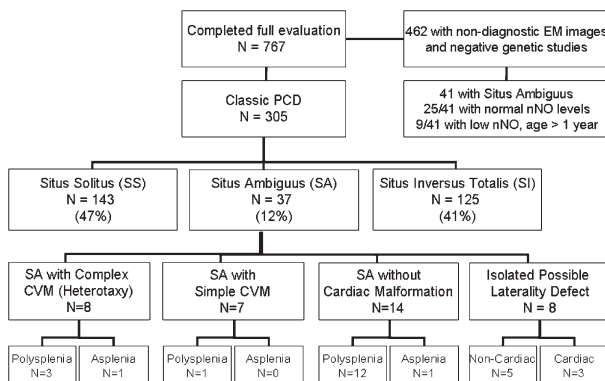


Figure 2 – Distribution of participants across laterality defect groups and situs ambiguus subgroups. CVM = cardiovascular malformation, EM = electron microscopic; nNO = nasal nitric oxide; PCD = primary ciliary dyskinesia.

TABLE 2] Demographics of Participants With SA Aged > 1 Year

Demographic or Laterality Defect	Classic PCD (n = 35)	Not Classic PCD, nNO Above Cutoff ^a (SA Control Group) (n = 25)	Not Classic PCD, nNO Below Cutoff ^a (n = 9)	P Value ^b
Age, y	12.4 (1.8-58)	5.6 (1.2-39)	8.0 (6.5-26)	.003 ^c
Male sex	34	56	56	.20
White race	80	83	78	.92
Chest radiograph for review	100	92	100	.17
CT/MRI scan chest for review	91	72	89	.13
Abdominal CT or ultrasound scan for review	89	76	89	.41
Echocardiogram for review	80	92	100	.18
Any congenital heart disease	49	68	78	.15
Classic heterotaxic heart defect	20	20	44	.31
Atrial situs inversus	26	32	22	.80
Common atrium	0	4	0	.41
Dextrocardia	46	50	33	.31
AVSD	9	8	44	.01 ^d
ASD	20	32	22	.56
VSD	26	40	0	.07
Single ventricle	9	12	11	.91
Double-outlet right ventricle	14	4	22	.26
Pulmonary stenosis or atresia	11	32	33	.11
Dextro-transposition of great arteries	3	24	11	.04 ^e
I-TGA	11	8	22	.46
Bilateral superior vena cava	6	21	11	.21
Right aortic arch	46	36	56	.56
Interrupted/duplicated inferior vena cava	34	39	22	.66
APVR	11	12	33	.23
Intestinal malrotation	24	21	44	.36
Dextrogastria	65	52	44	.56
Midline liver	21	21	33	.70
Asplenia	6	11	43	.03 ^f
Polysplenia	55	53	43	.85

Data are presented as median (range) or %. nNO = nasal nitric oxide; PCD = primary ciliary dyskinesia. See Table 1 legend for expansion of other abbreviation.

^aVelum closure nNO cutoff = 77 nL/min; tidal breathing nNO cutoff = 40 nL/min.

^b χ^2 test among three categorical groups.

^cKruskal-Wallis test for median age three-group comparison. Median age of classic PCD vs SA control group Wilcoxon $P = .002$; median age of classic PCD vs not classic PCD, nNO below cutoff, $P = .12$.

^dAVSD in classic PCD vs SA control group Fisher exact $P = .65$; in classic PCD vs not classic PCD, nNO below cutoff, $P = .02$.

^eDextro-transposition of the great arteries in classic PCD vs SA control group Fisher exact $P = .02$; in classic PCD vs not classic PCD, nNO below cutoff, $P = .38$.

^fAsplenia in classic PCD vs SA control group Fisher exact $P = .49$; in classic PCD vs not classic PCD, nNO below cutoff, $P = .03$.

and SA control participants (range healthy control participants, 125-867 nL/min; Kruskal-Wallis $P < .001$) (Fig 3). There were no significant differences in velum closure nNO levels among the SA subgroups with classic PCD (Kruskal-Wallis $P = .30$) (Fig 4).

Discussion

SI has been associated with PCD since the first case reports; however, SA with PCD has received attention only recently.^{15,16,27,28} This study reveals that the prevalence of SA (including heterotaxy) in a cohort of patients

TABLE 3] Diagnostic Testing of Participants With Classic PCD and SA

ID	Age, y	nNO, nL/min	Situs	EM Defect	Biallelic Mutation ^a
PCD1	0.1 ^b	8.79 ^c	SA + complex CVM	ODA + IDA	<i>HEATR2</i>
PCD2	0.2 ^b	11.2 ^c	SA + simple CVM	ODA + IDA	...
PCD3	1.8	16.1 ^c	SA + complex CVM	ODA	...
PCD4	3.0	4.6 ^c	SA + complex CVM	Indeterminate	<i>DNAH5</i>
PCD5	4.7	6.1 ^c	SA + simple CVM	ODA	...
PCD6	7.8	10.9 ^c	SA without cardiac defect	Indeterminate	<i>CCDC39</i>
PCD7	5.2	4.3	SA + complex CVM	Inadequate sample	<i>DNAH5</i>
PCD8	6.4	Not done	SA without cardiac defect	ODA	<i>DNAH5</i>
PCD9	7.3	6.8	SA + simple CVM	IDA + CA, MTD	<i>CCDC40</i>
PCD10	7.4	5.7	SA + complex CVM	ODA	<i>DNAH5</i>
PCD11	8.1	22.0	SA without cardiac defect	ODA	...
PCD12	8.1	10.4	SA + simple CVM	ODA + IDA	<i>DYX1C1</i>
PCD13	8.5	9.6	SA + simple CVM	IDA + CA, MTD	<i>CCDC40</i>
PCD14	9.3	10.0	SA without cardiac defect	ODA	...
PCD15	10.7	35.7	SA + simple CVM	ODA	<i>DNAH5</i>
PCD16	11.1	25.6	SA without cardiac defect	ODA + IDA	...
PCD17	11.2	9.8	SA without cardiac defect	IDA + CA, MTD	...
PCD18	11.4	10.2	Isolated possible laterality defect	ODA + IDA	<i>KTU</i>
PCD19	11.5	13.2	SA + complex CVM	ODA	<i>DNAI1</i>
PCD20	12.4	25.5	SA + complex CVM	Normal	<i>DNAH11</i>
PCD21	12.9	8.9	SA + complex CVM	Inadequate sample	<i>DNAH5</i>
PCD22	13.4	20.7	Isolated possible laterality defect	ODA + IDA defects	<i>HEATR2</i>
PCD23	14.0	32.5	Isolated possible laterality defect	ODA defect	<i>DNAH5</i>
PCD24	15.6	35.8	SA without cardiac defect	ODA defect	<i>DNAH5</i>
PCD25	16.0	11.9	Isolated possible laterality defect	IDA + CA, MTD	...
PCD26	16.9	16.5	SA without cardiac defect	ODA	...
PCD27	18.6	26.4	Isolated possible laterality defect	ODA	<i>DNAH5</i>
PCD28	20.5	29.9	SA + simple CVM	ODA	...
PCD29	22.7	5.5	Isolated possible laterality defect	IDA + CA, MTD	...
PCD30	24.6	35.7	Isolated possible laterality defect	ODA	<i>DNAH5</i>
PCD31	30.7	5.8	SA without cardiac defect	ODA	...
PCD32	31.7	13.1	SA without cardiac defect	ODA+IDA	<i>LRR6</i>
PCD33	34.7	47.6	SA without cardiac defect	ODA	<i>DNAH5</i>
PCD34	35.8	43.7	SA without cardiac defect	ODA	...
PCD35	45.8	12.1	SA without cardiac defect	ODA	<i>DNAH5</i>
PCD36	55.2	5.7	Isolated possible laterality defect	ODA	<i>DNAH5</i>
PCD37	58.0	7.2	SA without cardiac defect	ODA + IDA	...

CA = central apparatus; IDA = inner dynein arm; ODA = outer dynein arm; MTD = microtubule disorganization. See Table 1 and 2 legends for expansion of other abbreviations.

^aEllipses denote no genetic mutations isolated. We tested *DNAI1* and *DNAH5* for patients with ODA defects; *DNAAF2*, *DYX1C1*, *HEATR2*, and *LRR6* for patients with ODA + IDA defects; *CCDC39* and *CCDC40* for patients with IDA + CA and MTD defects; *DNAH11* for patients with normal ciliary ultrastructure; and all of these genes for patients in whom ciliary ultrastructure was not available.

^bParticipants aged <1 y not included in data analysis.

^cnNO values obtained with the tidal breathing technique.

TABLE 4] Clinical Features of Participants With SA Aged > 1 Year

Clinical Feature	Classic PCD (n = 35)	Not Classic PCD nNO Above Cutoff ^a (SA Control Group) (n = 25)	Not Classic PCD nNO Below Cutoff ^a (n = 9)	P Value ^b
Respiratory symptom				
Year-round wet cough	34 of 35 (97)	9 of 21 (43)	7 of 9 (78)	< .001 ^c
Year-round nasal congestion	33 of 35 (94)	16 of 23 (70)	6 of 8 (75)	.05 ^d
Digital clubbing	7 of 33 (21)	0 of 22 (0)	1 of 8 (13)	.07 ^e
NRD ^f	24 of 29 (83)	8 of 19 (42)	6 of 6 (100)	.001 ^g
Heart disease known to cause NRD	15 of 28 (54)	13 of 19 (68)	5 of 7 (71)	.38
Chronic otitis media	30 of 34 (88)	18 of 23 (78)	7 of 9 (78)	.58
Sinusitis	24 of 34 (71)	18 of 22 (82)	6 of 8 (75)	.59
Pneumonia/bronchitis	27 of 35 (77)	18 of 22 (82)	8 of 8 (100)	.32
Age of onset for respiratory symptom				
Year-round wet cough	0.08 (0.08-13)	0.59 (0.08-4)	0.33 (0.08-3)	.81
Year-round nasal congestion	0.08 (0.08-11)	0.42 (0.08-15)	1 (0.08-2)	.07 ^h
First episode otitis media	0.42 (0.08-5)	0.29 (0.08-8)	0.5 (0.25-23)	.98

Data are presented as No. of total No. (%) participants with indicated respiratory symptom or median age in y (range). NRD = neonatal respiratory disease. See Table 1 and 2 legends for expansion of other abbreviations.

^aVelum closure nNO cutoff = 77 nL/min; tidal breathing nNO cutoff = 40 nL/min.

^b χ^2 test among three groups.

^cCough in classic PCD vs SA control group Fisher exact $P < .001$; in classic PCD vs not classic PCD, nNO below cutoff, $P = .10$.

^dNasal congestion in classic PCD vs SA control group Fisher exact $P = .015$; in classic PCD vs not classic PCD, nNO below cutoff, $P = .15$.

^eClubbing in classic PCD vs SA control group Fisher exact $P = .021$; in classic PCD vs not classic PCD, nNO below cutoff, $P = .50$.

^fNot attributable to preterm birth or meconium aspiration.

^gNRD in classic PCD vs SA control group Fisher exact $P = .009$; in classic PCD vs not classic PCD, nNO below cutoff, $P = .37$.

^hAge of onset for nasal congestion in classic PCD vs SA control group Wilcoxon $P = .026$; in classic PCD vs not classic PCD, nNO below cutoff, $P = .44$.

with classic PCD is 12.1%, and this increases to 12.8% when excluding radial spoke and CA defects from an overall PCD denominator because these defects are not present in embryonic nodal cilia and, thus, do not present with heterotaxy. PCD is diagnosed in patients with various combinations of laterality defects, including simple and complex heart disease and combinations of noncar-

diac laterality defects, which do not fit into standard definitions of heterotaxy. Moreover, collections of laterality defects in some patients with SA do not correspond to the phenotypes of left or right isomerism, which often are used to group laterality defects clinically. The participants with confirmed PCD and SA without classic heterotaxic heart defects or isomerism sequence may

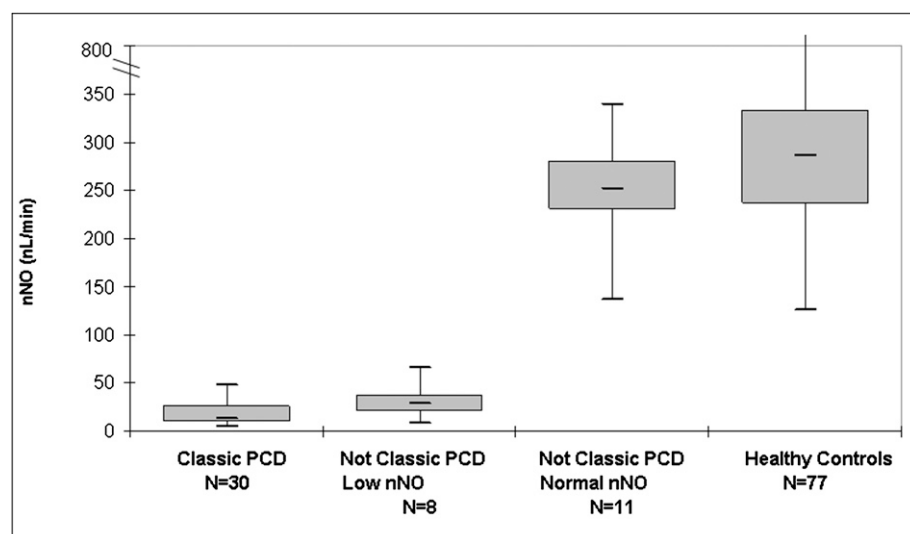


Figure 3 – Measurements of nNO in participants by final diagnosis group. Shown are nNO values by velum closure in participants with SA. Box plots show interquartile range, with median denoted by bold line. Whiskers denote minimum to maximum values. See Figure 2 legend for expansion of abbreviations.

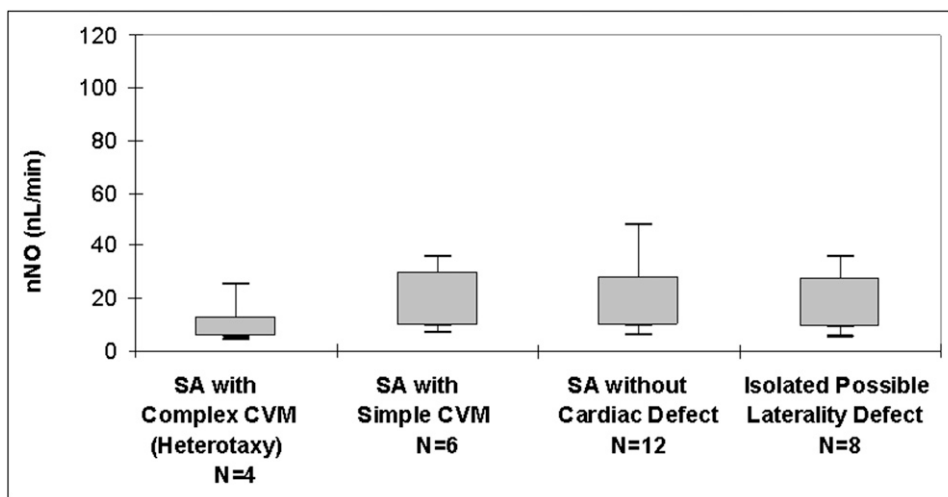


Figure 4 – Measurements of nNO in SA subgroups with classic primary cilia dyskinesia. Shown are nNO values by velum closure in participants with SA according to SA subgroup. Box plots show interquartile range, with median denoted by bold line. Whiskers denote minimum to maximum values. SA = situs ambiguus. See Figure 2 legend for expansion of other abbreviations.

represent a variant form of heterotaxy that is not fully appreciated.^{9,29,30} Genetic defects known to cause classic heterotaxy can also cause isolated d-TGA or double-outlet right ventricle, which in isolation are not considered heterotaxy by many cardiologists.³¹ Furthermore, isolated cardiac septal defects have been linked to ciliary genes.³² Thus, a wide spectrum of laterality defects may be attributable to ciliary dysfunction and associated with PCD.

The SA prevalence within the present PCD population is nearly twice that reported in the Kennedy et al¹⁵ study. Although our subclassification schema for SA was different from that of Kennedy et al,¹⁵ our definition of PCD and overall definition of laterality and cardiac defects comprising SA were similar. The increased prevalence in the present study may be due to heightened awareness of the link between heterotaxy and PCD, leading to increased referral patterns. Additionally, our use of standardized questions on laterality and cardiovascular anomalies within this prospective protocol may have been more attuned to the discovery of laterality defects than the retrospective protocol of Kennedy et al.¹⁵

No specific laterality defects were more prevalent in participants with classic PCD than in SA control participants. d-TGA was significantly more prevalent in SA control participants with normal nNO levels, yet one participant with d-TGA had classic PCD. Asplenia and atrioventricular septal defect, which often are seen together in right isomerism, were more prevalent in those without classic PCD and nNO levels below our cutoff. It is possible that these individuals carry an undiscovered PCD genetic mutation associated with right isomerism and normal

findings on electron microscopy. Perhaps with larger SA populations, one could find certain laterality defects associated with classic PCD.

Clinical symptom assessment in this SA population demonstrates that year-round wet cough and year-round nasal congestion were significantly more prevalent in participants with classic PCD than in SA control participants. Although one would expect a large proportion of patients with SA to have neonatal respiratory distress due to an increased burden of congenital heart disease, the prevalence of neonatal respiratory distress in participants with classic PCD was significantly more than in SA control participants. Therefore, in patients with forms of SA sparing the heart or with neonatal respiratory distress out of proportion with their cardiac defect, one should suspect possible PCD. Finally, digital clubbing was significantly more prevalent in participants with classic PCD than in SA control participants, even though clubbing is commonly found in patients with cyanotic congenital heart disease.³³

nNO is a useful test for PCD screening, but this has not been broadly studied in patients with SA. We saw significantly lower nNO levels in participants with SA and classic PCD than in SA control participants. All participants with SA and classic PCD had nNO levels below the previously established PCD cutoff of 77 nL/min.²⁰ Levels of nNO did not vary greatly among SA subgroups with classic PCD, which further supports the concept of SA as a spectrum of laterality defects with similar respiratory ciliary dysfunction regardless of the SA defects. A substantial number of participants had some features

suggestive of PCD with nNO levels below our cutoff value; however, they did not meet our criteria for classic PCD. With further genetic discovery, some of these may ultimately be defined as PCD. Until a full spectrum of PCD gene mutations is defined, clinicians may encounter patients with some clinical features of PCD and low nNO levels who do not fulfill classic definitions of PCD, as we saw here.

There are several limitations to this study. First, there is no universally accepted definition of heterotaxy or SA. Therefore, we used a classification schema that delineates patients with simple or complex heterotaxic heart defects, who often are followed by cardiologists, from others with noncardiac laterality defects and isolated possible laterality defects, who often are followed outside of cardiology. Some patients with SS or SI in our protocol did not have complete abdominal imaging. Consequently, some abdominal laterality defects may not be identified, and the numbers of participants with SA and classic PCD may be greater than reported here. Additionally, we did not use immunofluorescent ciliary staining, ciliary beat frequency, or functional ciliary analysis in our diagnostic algorithm. Conceivably, these techniques may have detected more cases of PCD, and we may have excluded PCD cases with normal ciliary ultrastructure and genetics but abnormal ciliary function. Second, the SA control group may not have been a pure control group because genetically proven PCD has been reported with normal ciliary ultrastructure³⁴ or

rarely with normal nNO levels.³⁵ All SA control participants were referred for chronic sino-oto-pulmonary symptoms, and a healthy SA control group may yield different results. Finally, SA control participants were significantly younger at study enrollment. Because the link between PCD and SA was recently discovered, physicians currently managing patients with SA may be more attuned to the presence of chronic respiratory symptoms in this population. With this knowledge, a higher burden of viral infections causing frequent sinusitis and otitis in young patients with SA may have prompted younger referrals to our consortium. This age disparity may also account for difference in sinopulmonary symptom prevalence because symptom onset may be delayed until an older age. However, this seems unlikely, because most patients with classic PCD exhibit sinopulmonary symptoms at birth and in the first year of life.

Conclusions

Understanding the potential for PCD in patients with laterality defects aside from SI is essential to improve clinical outcomes. Year-round wet cough, year-round nasal congestion, neonatal respiratory distress, and digital clubbing suggest PCD in patients with SA. Any patient with these symptoms and laterality defects apart from SI should have their respiratory cilia studied. Appropriate testing for PCD with SA includes genetic testing, nNO measurement, and EM ciliary examination at an experienced PCD center.

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Additional information: The e-Appendix and e-Table can be found in the Supplemental Materials section of the online article.

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