Primary ciliary dyskinesia (PCD) is a genetically heterogeneous, autosomal recessive disorder, with a prevalence of ≈1 in 15 000.1,2 Clinical disease reflects defective ciliary structure and function, and includes respiratory distress in term neonates, recurrent sinopulmonary infection, chronic otitis media, subfertility, and bronchiectasis.2 Diagnosis is usually confirmed by studies of ciliary function and ultrastructure, but more recently diagnosis has been facilitated by immunohistochemistry of cilia and measurements of nasal nitric oxide.2,3 Mutations that cause disease have been identified in 2 genes (DNAI1 and DNAH5) that code for ciliary outer dynein arm (ODA) proteins.4,5 Mutations in these genes are found in ≈35% of all PCD patients, and in as many as 60% of PCD patients with defects in the ciliary ODA.4,5

Abnormalities of thoraco-abdominal asymmetry occur in ≈50% of PCD patients.1,2 The organs are usually a mirror image of normal, which is situs inversus totalis (SI; Kartagener’s syndrome in PCD). SI occurs as a random phenomenon in PCD and reflects a loss of nodal ciliary function during embryogenesis.6–9

The recognition of heterotaxy in several PCD patients provoked more careful consideration of situs abnormalities in PCD. There is no consensus on the definition and classification of heterotaxy. Some authors use “situs ambiguus” and “heterotaxy” interchangeably as any abnormality of thoraco-abdominal asymmetry other than SI, whereas other authors suggest that “heterotaxy” includes 2 groups, SI and situs ambiguus.10,11
There are a number of recognized subtypes of heterotaxy, which are determined on the basis of cardiac atrium anatomy; left (polysplenia syndrome) and right (asplenia syndrome) disorders of isomerism sequence. The anatomic abnormalities associated with left and right isomerism have been described, but an unusual combination of anatomic abnormalities can make it difficult to classify as left or right isomerism.

An important consequence associated with heterotaxy is complex cardiac defects. Heterotaxy, which includes L-transposition of the great arteries, is associated with at least 3% of congenital heart disease (CHD). Earlier reports, which include autopsy series, suggested that the majority of patients with isomerism died in childhood because of CHD; however, interpretation of these reports alone leads to selection bias. In contrast, patients with left and right isomerism have been identified with no functional cardiac defect and a normal life span. Currently, ≈50% of patients with left isomerism with CHD survive to age 15.

Although a few case reports have noted that some PCD patients have heterotaxy, which includes CHD, polysplenia, and asplenia syndromes, the prevalence of these anatomic variations in PCD is not known. We reviewed a large cohort of PCD patients to determine the prevalence of heterotaxy and CHD and to define the types of ciliary defects and genetic mutations in DNAI1 and DNAH5 among these patients.

Methods

Patients
We performed a retrospective analysis of clinical and radiographic data of 337 PCD patients from the United States (n=147), Germany (n=128), Canada (n=36), and Australia (n=26) to identify patients with heterotaxy. Studies were performed under the auspices of respective Committees on the Protection of Rights to Human

Figure 1. Situs anomalies in 337 patients with primary ciliary dyskinesia.

Figure 2. Defining situs anomalies in PCD with chest x-ray. Posterior-anterior chest radiographs of 47-year-old monozygotic twins with PCD demonstrate situs solitus (A) and situs inversus totalis (B). C, Posterior-anterior chest radiograph of a 30-year-old female with PCD demonstrates abdominal situs inversus. Genotyping identified 1 DNAH5 and no DNAI1 mutation. D, Posterior-anterior chest radiograph of a 4-year-old male with PCD demonstrates isolated dextrocardia. H indicates heart apex; L, liver; S, stomach. Reproduced from Noone et al with permission from Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc. Copyright 1999.
Subjects. The diagnosis of PCD was confirmed by the presence of a compatible clinical phenotype and at least 1 confirmatory test, including (1) diagnostic abnormalities of ciliary structure by electron microscopy or mislocalization of axonemal dynein proteins with high-resolution immunofluorescence analysis (n=250), (2) abnormal ciliary beat pattern with high-speed video microscopy (n=56), and/or (3) nasal nitric oxide measurement (n=134). EM technique and interpretation, and high resolution immunofluorescence analysis of axonemal proteins, have been described. Nasal production of nitric oxide was measured at 2 sites (USA and Canada), using either a Siever system (normal mean=±1 SD=376±124 nL/min) or a NIOX system (normal=400 to 1000 ppb). Genetic analyses of DNAI1 and DNAH5 had been previously performed in 161 subjects.

Methodology to Identify Heterotaxy

Patients were ultimately subdivided into 3 distinct groups: situs solitus (SS; normal thoraco-abdominal asymmetry), SI (complete mirror image reversal of SS with no other defect) and heterotaxy (situs ambiguous [SA]; any thoracoabdominal asymmetry that differs from SS or SI). Heterotaxy was subdivided into 3 groups: left isomerism, right isomerism, and other (including isolated dextrocardia and abdominal SI). To define situs status, we used the chest x-ray for preliminary assessment: SS (left-sided cardiac apex and stomach bubble), SI (right-sided cardiac apex and stomach bubble), isolated dextrocardia (right-sided cardiac apex and left-sided stomach bubble), and abdominal SI (left-sided cardiac apex and right-sided stomach bubble). To further determine classification of heterotaxy, we reviewed available computed tomography, magnetic resonance imaging, and abdominal sonographic and echocardiographic studies. If available studies did not allow classification, patients were contacted again to obtain relevant radiographic studies and surgical reports. Polysplenia was identified when the splenic mass was divided into fairly equal-sized masses that varied in number from 2 to 6 and ranged from 1 to 6 cm in diameter, which together approximated the mass of a normal spleen.

Results

Situs Status

Of 337 PCD patients, SS was present in 46.0% and SI in 47.7%; in addition, 6.3% (21 patients) had heterotaxy (Figures 1 and 2).

The imaging methodology used to classify these patients by situs group is summarized in Table 1. More than 65% and 76% of patients classified as SI or heterotaxy, respectively, had echocardiograms and abdominal imaging.

Ciliary Defects

There is a clear shift in the types of ciliary defects across the 3 groups classified by situs status (P<0.001) (Table 2). Specifically, there was a decreasing prevalence of inner dynein arm and central apparatus ciliary defects, as well as an increasing prevalence of ODA defects from SS to SI to heterotaxy. The higher prevalence of ODA defects in patients with situs abnormalities (SI plus heterotaxy) was strikingly different from SS (P<0.001). This indicates that ODA defects are more commonly involved (and inner dynein arm and/or central defects are less commonly involved) in PCD patients with abnormalities of organ development or location.

Statistical Analysis

For the primary analysis, Fisher exact test was used to test for differences in the prevalence of 3 different types of ciliary defects and prevalence of mutations in DNAI1 and DNAH5 across groups of patients with SS, SI, and heterotaxy. Secondary analyses used Fisher exact test to compare pairs of situs groups (SS, SI, SA) and to compare patients with situs abnormalities to those with SS. A probability value of <0.05 was considered significant for all analyses; no adjustment was made for multiple comparisons.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

TABLE 1. Imaging Used to Classify Heterotaxy in 337 Patients With Primary Ciliary Dyskinesia

<table>
<thead>
<tr>
<th>Imaging Method</th>
<th>Situs Solitus (n=155)</th>
<th>Situs Inversus Totalis (n=161)</th>
<th>Heterotaxy (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest x-ray</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Echocardiogram</td>
<td>23</td>
<td>65</td>
<td>76</td>
</tr>
<tr>
<td>Thoracic CT</td>
<td>33</td>
<td>40</td>
<td>86</td>
</tr>
<tr>
<td>Abdominal imaging*</td>
<td>53</td>
<td>66</td>
<td>95</td>
</tr>
</tbody>
</table>

Values expressed as %. CT indicates computed tomography.

*CT thorax with upper abdominal images, CT abdomen, ultrasound abdomen, magnetic resonance imaging of abdomen, or surgical findings.

TABLE 2. Types of Ciliary Defects and Genetic Mutations in 3 Groups of PCD Patients Who Had Characterization of Ciliary Defect and Genetic Analyses of 2 Cilia Genes (DNAI1 and DNAH5)

<table>
<thead>
<tr>
<th>Ciliary Defect (n=250 patients)*</th>
<th>Situs Solitus (n=122)</th>
<th>Situs Inversus Totalis (n=112)</th>
<th>Heterotaxy (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer dynein arm defect (n=165)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner dynein arm defect, alone (n=69)</td>
<td>46</td>
<td>37.7</td>
<td>18.8</td>
</tr>
<tr>
<td>Central apparatus defect (n=16)§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutations in DNAI1 or DNAH5 (n=60¶)</td>
<td>19</td>
<td>27.1</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher exact test (P<0.001). Types of ciliary defects differed across the 3 situs groups.
†Of these 165 patients with outer dynein arm defects, 57 also had some inner dynein arm defect (n=24, 27, and 6 for situs solitus, situs inversus, and heterotaxy, respectively).
‡Fisher exact test. Different from situs inversus (P<0.001); different from heterotaxy (P=0.008); different from situs inversus plus heterotaxy (P<0.001).
§Central pair microtubule defect or radial spoke defect.
¶At least 1 mutant allele (see text for details).
#Fisher exact test (P=0.022). Different from situs inversus plus heterotaxy.
### Genetic Mutations

Genetic testing of 161 PCD patients for mutations in 2 ciliary ODA genes (DNAI1 and DNAH5) paralleled the pattern of increasing prevalence of ciliary ODA defects across the 3 situs groups (P = 0.037) (Table 2). Specifically, there was an increasing prevalence of mutations from SS to SI to heterotaxy. The higher prevalence of mutations in patients with situs abnormalities (SI plus heterotaxy) was different from SS (P = 0.022). The distribution of DNAI1 versus DNAH5 mutations were similar across the 3 situs groups, as were the number of mutated alleles (patients with 2 mutations ranged from 71% to 84% in the 3 situs groups), and types of mutation (frameshift/STOP alleles ranged from 83% to 92%).

### Heterotaxy Patients

There was an equal distribution of gender (11 females/10 males), and the mean age was 17 (range 1 to 54) years (Table 3). The clinical phenotype was consistent with PCD in all heterotaxy patients; 76% had respiratory distress as term neonates, and all adults (age ≥18 years) and 50% of pediatric patients (age <18 years) had bronchiectasis. Sixteen of the heterotaxy patients had characterization of the ciliary defect, and 14 patients had an ODA defect. Nasal nitric oxide was low in the 11 patients tested, consistent with PCD. Seven of 12 patients tested had at least 1 mutation in DNAI1 or DNAH5, and 5 patients had 2 mutations. Three patients with heterotaxy had siblings with PCD (2 with Kartagener’s syndrome) (Table 3). Another patient (UNC927) had a brother born preterm (32 weeks) who died at 2 days of life with left isomerism (polysplenia) and CHD. Other clinical features included pectus excavatum (2 of 21 patients) as per the Haller Index (Table 4). Intestinal malrotation (left-sided appendectomy) was identified in only 1 patient (UNC875).

### Laterality Defect Subtypes

The distribution of heterotaxic subtypes in 21 PCD patients is illustrated (Figure 1) and anatomic findings are summarized (Table 4). Eleven patients had left isomerism, which included 6 patients with polysplenia and cardiac and/or vascular anomalies (3 patients had CHD) (Figure 3, Table 4). One patient had right isomerism (Figure 4). Nine patients had other heterotaxic anomalies (3 patients with SI plus CHD; 3 patients with abdominal SI; 2 patients with isolated dextrocardia; and 1 patient with SS with CHD) (Figure 2). Five patients had polysplenia, but without cardiovascular anomalies (2 patients with abdominal SI, 1 patient with SI, and 2 patients with SS).

### Cardiac and/or Vascular Malformations

Twelve of the 21 PCD patients with heterotaxy had cardiac and/or vascular malformations. Four of these 12 patients had vascular anomalies alone (Tables 4 and 5), and 8 patients had complex cardiac anomalies that required surgery. In 3 patients with CHD and left isomerism, the cardiac defects included double outlet right ventricle and atrioventricular...
canal defects. In another 3 patients with CHD and SI, there were atrial and ventricular septal defects and L-transposition of the great arteries. Of the other 2 patients with CHD, one had abdominal situs inversus and atrial and ventricular septal defects, and 1 patient had SS and tetralogy of Fallot. There were 8 other patients with left isomerism who did not have complex cardiac malformations.

**Discussion**

The phenotype of PCD may be confused with other diseases, and diagnosis is often delayed.34 The presence of SI often aids diagnosis because of its association with PCD (Kartagener’s syndrome). However, even those patients with SI are frequently not diagnosed with PCD. Our retrospective study of laterality defects in a large PCD population indicates that heterotaxic anomalies are present in at least 6.3% of these patients. The prevalence (6.3%) is likely an underestimation, because echocardiogram and abdominal imaging are not routinely performed in PCD, and subtle anatomic abnormalities not recognized.

The classification of heterotaxy syndromes remains controversial.10,11,14 We attempted to characterize all heterotaxy (situs ambiguus) patients as right or left isomerism. However, 9 patients

**Table 4. Anatomy of 21 Patients With PCD and Heterotaxy**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Heterotaxy</th>
<th>Imaging</th>
<th>Spleen</th>
<th>Liver</th>
<th>Heart</th>
<th>Vascular</th>
<th>Pulmonary*</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNC373</td>
<td>RI</td>
<td>CT, E, A</td>
<td>ASP</td>
<td>Middle</td>
<td>Left</td>
<td>IVC int, AZVC</td>
<td>Normal</td>
<td>Dextrogastria, Bil DU, HH</td>
</tr>
<tr>
<td>UNC875</td>
<td>LI</td>
<td>CT, E, A</td>
<td>LPSP</td>
<td>Right</td>
<td>Left</td>
<td>IVC int, HAZVC, Bil SVC</td>
<td>Normal</td>
<td>Intestinal malrotation</td>
</tr>
<tr>
<td>G122</td>
<td>LI</td>
<td>CT, A</td>
<td>RSPP</td>
<td>Left</td>
<td>Left</td>
<td>Normal</td>
<td>Normal</td>
<td>Dextrogastria</td>
</tr>
<tr>
<td>G71</td>
<td>LI</td>
<td>CT, A</td>
<td>LPSP</td>
<td>Right</td>
<td>Left</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>UNC471</td>
<td>LI</td>
<td>CT, E, A</td>
<td>RPSP</td>
<td>Left</td>
<td>Left</td>
<td>Normal</td>
<td>Normal</td>
<td>Dextrogastria</td>
</tr>
<tr>
<td>UNC231</td>
<td>LI</td>
<td>CT, E, A</td>
<td>LPSP</td>
<td>Right</td>
<td>Left</td>
<td>Normal</td>
<td>Normal</td>
<td>Pect Exc HI=3.0</td>
</tr>
<tr>
<td>CN2</td>
<td>LI+ CHD</td>
<td>CT, E, A</td>
<td>LPSP</td>
<td>Right</td>
<td>Left, LAI</td>
<td>IVC int, AZVC</td>
<td>Bil Hypart</td>
<td>Dextrogastria</td>
</tr>
<tr>
<td>CN3</td>
<td>LI</td>
<td>CT, E, A</td>
<td>RSPP</td>
<td>Left</td>
<td>Right</td>
<td>Normal</td>
<td>Reversed</td>
<td>Dextrogastria</td>
</tr>
<tr>
<td>UNC930</td>
<td>LI+ CHD</td>
<td>CT, E, A</td>
<td>RSPP</td>
<td>Middle</td>
<td>Left, LAI, AVSD, DORV, SPS, HB</td>
<td>IVC int, AZVC, Bil SVC</td>
<td>Bil Hypart</td>
<td>Normal</td>
</tr>
<tr>
<td>G30</td>
<td>LI+ CHD</td>
<td>CT, E, A</td>
<td>LPSP</td>
<td>Right</td>
<td>Right, LAI, CA AVSD, DORV</td>
<td>ACOARC</td>
<td>Bil Hypart</td>
<td>Normal</td>
</tr>
<tr>
<td>UNC340</td>
<td>LI</td>
<td>CT, E, A</td>
<td>RSPP</td>
<td>Left</td>
<td>Right</td>
<td>IVC int, AZVC</td>
<td>Reversed</td>
<td>Dextrogastria</td>
</tr>
<tr>
<td>UNC927</td>
<td>LI</td>
<td>CT, E, A</td>
<td>RSPP</td>
<td>Right</td>
<td>Left</td>
<td>IVC int, AZVC</td>
<td>Normal</td>
<td>Pancreatic hypoplasia</td>
</tr>
<tr>
<td>CN4</td>
<td>Other: SS+CHD</td>
<td>CT, E, A</td>
<td>LPSP</td>
<td>Right</td>
<td>Left</td>
<td>Tetralogy of Fallot</td>
<td>Tetralogy of Fallot</td>
<td>Normal</td>
</tr>
<tr>
<td>G19</td>
<td>Other: ASI+CHD</td>
<td>CT, E, A</td>
<td>RSPP</td>
<td>Left</td>
<td>Left</td>
<td>ASD, VSD, LTGA</td>
<td>Normal</td>
<td>Bil Hypart</td>
</tr>
<tr>
<td>G725</td>
<td>Other: ASI</td>
<td>CT, A</td>
<td>RSPP</td>
<td>Left</td>
<td>Left</td>
<td>Normal</td>
<td>Normal</td>
<td>Dextrogastria</td>
</tr>
<tr>
<td>AU666</td>
<td>Other: ASI</td>
<td>Und</td>
<td>RSPP</td>
<td>Left</td>
<td>Left</td>
<td>Normal</td>
<td>Normal</td>
<td>Dextrogastria</td>
</tr>
<tr>
<td>G29</td>
<td>Other: IDEX</td>
<td>E, A</td>
<td>RSPP</td>
<td>Right</td>
<td>Right</td>
<td>Normal</td>
<td>Reversed</td>
<td>Normal</td>
</tr>
<tr>
<td>G103</td>
<td>Other: IDEX</td>
<td>A</td>
<td>RSPP</td>
<td>Right</td>
<td>Right</td>
<td>Normal</td>
<td>Reversed</td>
<td>Normal</td>
</tr>
<tr>
<td>UNC919</td>
<td>Other: SI+CHD</td>
<td>CT, E, A</td>
<td>RSPP</td>
<td>Right</td>
<td>Right, VSD, LVOTob, SPS, LTGA</td>
<td>Normal</td>
<td>Reversed</td>
<td>Dextrogastria</td>
</tr>
<tr>
<td>G246</td>
<td>Other: SI+CHD</td>
<td>CT, E, A</td>
<td>RSPP</td>
<td>Right</td>
<td>Right</td>
<td>LGTA</td>
<td>Normal</td>
<td>Reversed</td>
</tr>
<tr>
<td>UNC1005</td>
<td>Other: SI+CHD</td>
<td>CT, E, A</td>
<td>RSPP</td>
<td>Right</td>
<td>Right</td>
<td>ASD, VSD</td>
<td>Normal</td>
<td>Reversed</td>
</tr>
</tbody>
</table>

CT indicates computed tomography of chest; A, abdominal imaging (see text for details); ACOARC, aortic coarctation; ASD, atrial septal defect; ASI, abdominal situs inversus; ASP, asplenia; AVSD, atrioventricular septal defect; AZVC, azygos vein continuation; Bil, bilateral; CA, common atrium; DORV, double outlet right ventricle; DU, duplicate ureters; E, echocardiogram; HAZVC, hemiazygos vein continuation; HB, heart block; HI, hallus index (normal 2.433); HI, heart block; HH, hiatal hernia; Hypart, hyparterial bronchi, bilobed lungs; IDEX, isolated dextrocardia; IVC int, inferior vena cava interruption; LAI, left atrial isomerism; L, left isomerism; LPSP, left-sided polysplenia; LTGA, L-transposition of great arteries; LVOTob, left ventricular outflow tract obstruction; Pect Exc, pectus excavatum; HI, Hallier Index (normal <2.4)13; RI, right isomerism; RPSP, right-sided polysplenia; SPS, subpulmonic stenosis; SVC, superior vena cava; Und, undefined; and VSD, ventricular septal defect.

*Excludes pathology related to chronic airway disease.

**Figure 3.** Left isomerism with polysplenia. Contrast-enhanced computed tomography scan of a 41-year-old male with PCD demonstrates features of left isomerism with polysplenia. A. Note bilateral superior vena cava at the level of aortic arch (a). Right superior vena cava is enhanced after intravenous contrast material administration through a right antecubital vein. Left-sided superior vena cava indicated by white arrow. B. Upper abdomen image demonstrates left upper quadrant splenules (s).
could not be characterized, despite abdominal and echocardiogram imaging in most of these patients. We classified 3 patients with SI and congenital heart disease as heterotaxy, because these patients did not have mirror image reversal of situs solitus. One patient classified as having right isomerism (asplenia, midline liver) had inferior vena cava interruption, although inferior vena cava interruption is unusual in right isomerism.\(^{17,19,35}\)

The respiratory phenotypes of the PCD patients with heterotaxy match those without heterotaxy.\(^2\) Specifically, the majority of patients had respiratory distress at birth, typical sinopulmonary disease, and chronic otitis media (Table 3). Bronchiectasis was present in all adults and \(\approx 50\%\) of children.\(^{2,36,37}\)

Strikingly, there was a 200-fold higher prevalence of CHD related to heterotaxy in PCD (1 in 50 patients) versus the general population (CHD related to heterotaxy: 1 in 10 000 patients).\(^38\) PCD is not routinely cited as a cause of CHD.\(^39,40\) We speculate that the diagnosis of PCD is not made in many PCD patients who also have heterotaxy and CHD. For instance, the diagnosis of PCD was not made in 1 patient (UNC930) until age 10 years and after cardiac surgery. The diagnosis of PCD must be considered in patients with CHD and heterotaxy, particularly in patients with recurrent respiratory symptoms. Conversely, patients diagnosed with PCD should have formal cardiac assessment, especially patients with heterotaxic anatomic defects such as polysplenia.

More than half the PCD patients with heterotaxy had polysplenia (left isomerism; 11 of 21 patients), and 1 additional patient had asplenia. A spectrum of anatomic anomalies was seen in PCD patients with polysplenia or asplenia, which ranged from splenic anomaly alone to vascular anomalies to CHD and lung isomerism. The severity of polysplenia syndrome varied even within a single family, as 1 patient had vascular without cardiac anomalies, but a brother had complex CHD.

The establishment of left-right axis in the embryo is complex.\(^11\) Embryonic nodal dysfunction is 1 cause of randomization of left-right asymmetry and involves 2 distinct types of embryonic nodal cilia (motile and nonmotile sensory).\(^7,8,41\) In PCD, it is hypothesized that SI reflects defective nodal ciliary motile function,\(^4,6,42\) and our clinical data support that hypothesis; specifically, PCD patients can manifest heterotaxy, and siblings with PCD can have different situs anomalies. The spectrum of heterotaxic anomalies identified, such as CHD, is consistent with animal models of heterotaxy.\(^9,43–45\) In fact, 40% of mice with mutations in an

### TABLE 5. Cardiovascular Abnormalities in 12 Patients With Primary Ciliary Dyskinesia and Heterotaxy

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Left Isomerism (n=6)</th>
<th>Right Isomerism (n=1)</th>
<th>SS (n=1)</th>
<th>SI (n=3)</th>
<th>Abdominal SI (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic veins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral superior vena cava</td>
<td>2</td>
<td>0</td>
<td>0 0 0</td>
<td>0</td>
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</tr>
<tr>
<td>Inferior vena cava drainage via azygous</td>
<td>4</td>
<td>1</td>
<td>0 0 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Inferior vena cava drainage via hemiazygous</td>
<td>1</td>
<td>0</td>
<td>0 0 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Great vessels</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>0</td>
<td>0</td>
<td>1 0 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>L-transposition of the great arteries</td>
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<td>0</td>
<td>0 2 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic coarctation</td>
<td>1</td>
<td>0</td>
<td>0 0 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sub pulmonic stenosis</td>
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<td>0</td>
<td>0 1 0</td>
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<td></td>
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<tr>
<td>Intracardiac</td>
<td></td>
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<td></td>
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<tr>
<td>Left atrial isomerism</td>
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<td>Right atrial isomerism</td>
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<td>Atrial septal defect</td>
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<td>0 1 1</td>
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<td></td>
</tr>
<tr>
<td>Common atrium</td>
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axonal dynein heavy chain gene (*Ird*; iv/iv mice) show visceral, cardiac, and venous malformations of heterotaxy and small litter sizes because of intrauterine death.\(^{4,5}\)

The distribution of different types of ciliary defects and genetic mutations in PCD patients classified by situs status strongly support the concept that specialized embryonic nodal cilia play a key role in organ development and location. These nodal cilia are motile, even though they do not have the central apparatus (central pair microtubules or radial spokes); thus, the increasing prevalence of ODA defects (and decreasing prevalence of inner dynein arm and central apparatus defects) from SS to SI to heterotaxy is consistent with this concept.

Likewise, the distribution of mutations in 2 genes (*DNAI1* and *DNAH5*) that code for respiratory and nodal ciliary ODA proteins are consistent with this concept; ie, there is an increased prevalence of mutations from SS to SI to heterotaxy. Stated another way, genetic mutations that do not affect the outer ciliary microtubule doublets in the nodal cilia (such as central apparatus genes) are less likely to result in SI or heterotaxy. In support of this concept, mice deficient in *Mduh5* (murine homolog of *DNAH5*) also develop SI totalis and heterotaxy; these occur in association with ciliary immotility and recurrent respiratory infections.\(^{46,47}\) Thus, PCD phenotypes with heterotaxy, such as polysplenia and CHD, are frequently associated with mutations in *DNAI1* and *DNAH5*, and genetic testing is indicated in these patients. These observations broaden both the phenotypic spectrum of *DNAI1* and *DNAH5* mutations and our understanding of the genetic causes of heterotaxy, and have important clinical implications.

It is unclear why the prevalence of heterotaxy in PCD is only one-tenth as common as SS or SI, and it does not seem likely that the prevalence of heterotaxy would change substantially, even if all PCD patients underwent full radiographic and ultrasound imaging. It is possible that humans (like mice) with heterotaxy and life-threatening anomalies (such as CHD) may be dying in utero or in early life.\(^{45}\) Mutations in other as yet unidentified ciliary genes likely cause PCD and may have consequences for embryonic node function and left-right asymmetry pathways at other steps. Maternal diabetes, paternal cocaine use, and retinoic acid deficiency have also been associated with heterotaxy; however, we have no data on these confounders in our PCD patients.\(^{48}\)

A previously unrecognized anomaly was the high prevalence of pectus excavatum (~10%) in our PCD patients with heterotaxy compared with the general population (0.3%).\(^{49}\) Although 1 PCD patient with pectus had previous cardiac surgery, the pectus excavatum was documented prior to surgery. Interestingly, pectus excavatum and CHD are described in other congenital disorders, such as Marfan syndrome and Noonan syndrome, and an association between pectus excavatum and SI has previously been identified.\(^{50}\) At this point, it is uncertain if there is a genetic or pathophysiological link between pectus excavatum, heterotaxy, and PCD.\(^{51}\)

In conclusion, we demonstrate that PCD is associated with a marked increase in the prevalence of heterotaxy with and without CHD. The association of ciliary motility defects and heterotaxy links ciliary dysfunction and CHD. Thus, cilia-related genes are excellent candidate genes for heterotaxy and CHD. Patients with SI or heterotaxic anomalies, particularly those with concomitant neonatal respiratory distress or chronic respiratory infections, are at risk to have an underlying defect in ciliary structure, function, and genetics, and should be evaluated for PCD and tested for genetic mutations in *DNAI1* and *DNAH5*.

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Disclosures

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References

15. Ivermark BI. Implications of agenesis of the spleen on the pathogenesis of congenital anomalies in childhood: analysis of the heart malformations in the spleenic


**CLINICAL PERSPECTIVE**

Congenital heart disease (CHD) and abnormalities of organ anatomy and location (heterotaxy/situs ambiguus) are leading causes of morbidity in infants, and little is known about the underlying genetics of these disorders in humans. A few case reports have noted that some patients with primary ciliary dyskinesia (PCD), a genetic disorder of ciliary function, have heterotaxy with CHD and/or polysplenia, or asplenia syndromes. To better define the prevalence of these clinical disorders in PCD, we reviewed 337 well-characterized PCD patients from 4 specialized centers in the United States, Germany, Canada, and Australia. We determined that at least 6.3% (n=21) of these PCD patients had heterotaxy, which was largely associated with defects in the outer dynein arm of respiratory cilia and mutations in ciliary outer dynein arm genes (*DNAI1* and *DNAH5*). Twelve of these patients with heterotaxy had cardiac and/or vascular abnormalities, and most (8 of 12 patients) had complex CHD that required surgery. The prevalence of CHD with heterotaxy was noted to be 200-fold higher in PCD than in the general population (1:50 versus 1:10 000), which indicates that patients with PCD should have formal cardiac evaluation. Conversely, genetic mutations that adversely affect respiratory and embryological nodal cilia are a significant cause of heterotaxy and CHD, and these patients should be evaluated for PCD.
Congenital Heart Disease and Other Heterotaxic Defects in a Large Cohort of Patients With Primary Ciliary Dyskinesia

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