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Risk Factors for Situs Defects and Congenital Heart Disease in Primary Ciliary Dyskinesia

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Complete List of Authors:	Best, Sunayna; University College London Shoemark, Amelia; Royal Brompton Hospital, Electron Microscopy; Imperial College London, National Heart and Lung Institute Rubbo, Bruna; University of Southampton Faculty of Medicine, Clinical and Experimental Sciences Academic Unit Patel, Mitali; University College London Fassad, Mahmoud; University College London, Institute of Child Health; Alexandria University Medical Research Institute, Human Genetics Department Dixon, Mellisa; Royal Brompton Hospital, Electron Microscopy Unit Rogers, Andrew; Royal Brompton Hospital Hirst, Robert; University of Leicester, Primary Ciliary Dyskinesia Diagnostic Service, Department of Infection, Immunity and Inflammation, Robert Kilpatrick Clinical Sciences Building Rutman, Andrew; University of Leicester, Primary Ciliary Dyskinesia Diagnostic Service, Department of Infection, Immunity and Inflammation, Robert Kilpatrick Clinical Sciences Building Ollosson, Sarah; Royal Brompton and Harefield NHS Foundation Trust Jackson, Claire; University of Southampton Faculty of Medicine, Clinical and Experimental Sciences Academic Unit Goggin, Patricia; University Hospital Soutahmpton NHS Foundation Trust, Primary Ciliary Dyskinesia Centre Thomas, Simon; University of Southampton Faculty of Medicine, Human Genetics and Genomic Medicine Pengelly, Reuben; University of Southampton Faculty of Medicine, Human Genetics and Genomic Medicine Cullup, Thomas; North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children Pissaridou, Eleni; University College London Great Ormond Street Institute of Child Health, Population, Policy and Practice Programme Hayward, Jane; North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children Onoufriados, Alexandros; King's College London, Division of Genetics and Molecular Medicine O'Callaghan, Christopher; University College London, Respiratory, Critical Care & Anaesthesia Loebinger, Michael; Royal Brompton Hospital, Host Defence Unit, Division of Respiratory Medicine

	Wilson, Robert; Royal Brompton Hospital Chung, Eddie; University College London Kenia, Priti; Leicester Royal Infirmary, Children's Hospital Doughty, Victoria; Royal Brompton Hospital, Brompton Centre for Fetal Cardiology Carvalho, Julene; Royal Brompton Hospital, Brompton Centre for Fetal Cardiology Lucas, Jane; University of Southampton Faculty of Medicine, Clinical and Experimental Sciences Academic Unit; NIHR Southampton Respiratory Biomedical Research Unit, Southampton University Hospitals Trust Mitchison, Hannah; University College London Hogg, Claire; Royal Brompton Hospital, Paediatric Respiratory
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Risk Factors for Situs Defects and Congenital Heart Disease in Primary Ciliary Dyskinesia

Sunayna Best BSc MBBS MSc MRCPCH[1,2], Amelia Shoemark BSc PhD[2], Bruna Rubbo MBBS MSc[3,4], Mitali P. Patel BSc MSc[1], Mahmoud R. Fassad MBBCH MSc [1,5], Mellisa Dixon BSc PhD[2] Andrew V. Rogers BSc PhD[2], Robert A. Hirst BSc PhD[6], Andrew Rutman CBiol MRSB[6], Sarah Ollosson BSc[2], Claire Jackson BSc MSc PhD[3,4], Patricia Goggin BSc, MMedSci.[3,4], Simon Thomas BSc PhD[7,8], Reuben Pengelly MBiol PhD[7], Thomas Cullup BSc[9], Eleni Pissaridou BSc MSc[10], Jane Hayward BSc PhD[1,9], Alexandros Onoufriadis BSc MSc PhD[11], Christopher O'Callaghan BMedSci BM BS PhD DM[6,12], Michael R. Loebinger MA FRCP PhD[13], Robert Wilson MD FRCP[13], Eddie Chung MBChB MD FRCPCH[10], Priti Kenia MBBS, MD Paediatrics, MRCPCH[14], Victoria L. Doughty BSc PhD[15], Julene S. Carvalho MD PhD FRCPCH[15,16,17], Jane S. Lucas BM PhD [3,4], Hannah M. Mitchison BSc PhD[1*‡], Claire Hogg MBChB, BSc MRCPCH[2*]

[1]Genetics and Genomic Medicine, University College London, UCL Great Ormond Street Institute of Child Health, London WC1N 1EH, UK

[2] PCD Diagnostic Team, Department of Paediatric Respiratory Medicine, Royal Brompton and Harefield NHS Trust, Sydney Street, London SW3 6NP, UK

[3] Primary Ciliary Dyskinesia Centre, University Hospital Southampton NHS Foundation Trust and Clinical and Experimental Sciences Academic Unit, University of Southampton Faculty of Medicine, Southampton SO17 1BJ, UK

[4] NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, UK

[5] Human Genetics Department, Medical Research Institute, Alexandria University, Alexandria 21561, Egypt

[6] Centre for PCD Diagnosis and Research, Department of Infection, Immunity and Inflammation, RKCSB, University of Leicester, Leicester LE2 7LX, UK

[7] Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton SO17 1BJ, UK

[8] Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury District Hospital, Salisbury SP2 8BJ, UK

[9] North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children, London WC1N 3BH, UK

[10] Population, Policy and Practice Programme, University College London Great Ormond Street Institute of Child Health, 30 Guildford Street, London WC1N 1EH, UK

[11] Department of Medical and Molecular Genetics, Division of Genetics and Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London SE1 9RT, UK

[12] Respiratory, Critical Care & Anaesthesia, UCL Great Ormond Street Institute of Child Health, London WC1N 1EH, UK

[13] Host Defence Unit, Royal Brompton and Harefield NHS Trust, Sydney Street, London SW3 6NP, UK

[14] Department of Respiratory Paediatrics, Birmingham Children's Hospital NHS Foundation Trust, Steelhouse Lane, Birmingham B4 6NH, UK

[15] Brompton Centre for Fetal Cardiology, Royal Brompton and Harefield NHS Trust, Sydney Street, London, SW3 6NP, UK

[16] Fetal Medicine Unit, St George's University Hospitals NHS Foundation Trust, Blackshaw Road, Tooting, London SW17 0QT, UK

[17] Molecular & Clinical Sciences Research Institute, St George's, University of London, Cranmer Terrace, London SW17 0RE, UK

* Shared senior authorship

‡**Correspondence addresses:**

Dr Hannah Mitchison, Genetics and Genomic Medicine, UCL Institute of Child Health,

30 Guilford Street, London WC1N 1EH

Fax. +44 (0)20 7404 6191

Tel. +44 (0)20 7905 2866

Email. h.mitchison@ucl.ac.uk

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Abstract

Primary ciliary dyskinesia (PCD) is associated with abnormal organ positioning (situs) and congenital heart disease (CHD). This study investigated genotype-phenotype associations in PCD to facilitate risk predictions for cardiac and laterality defects. This retrospective cohort study of 389 UK PCD patients found 51% had abnormal situs and 25% had CHD and/or laterality defects other than situs inversus totalis. Patients with bi-allelic mutations in a subset of nine PCD genes all had normal situs. Patients with consanguineous parents had higher odds of situs abnormalities than patients with non-consanguineous parents. Patients with abnormal situs had higher odds of CHD and/or laterality defects.

Summary box

What is the key question?

What is the prevalence of situs, cardiac defects and other laterality defects amongst patients with PCD, and are there any significant clinical or genetic risk factors for these?

What is the bottom line?

Congenital heart disease and other laterality defects are significantly more prevalent in a cohort of 389 UK-based PCD patients than previously reported, with a clear subset of PCD genes not associated to situs abnormalities.

Why read on?

This is the first study investigating situs and laterality defects in PCD patients from the United Kingdom (UK) and the largest genotype-phenotype correlation study in PCD to date.

Introduction

Primary Ciliary Dyskinesia (PCD) arises from dysfunction of motile cilia and has an estimated prevalence of one in 10,000 births. Abnormal cilia structure or function leads to organ laterality defects in approximately half of PCD patients [1 2]. This arises due to impaired function of motile cilia in the embryonic left-right (LR) organiser (node) [3], causing random assignment of thoraco-abdominal orientation. Two past studies investigated rates of laterality defects and CHD in PCD, with combined results showing 3.5-6% of PCD patients had a cardiovascular malformation [4-6].

To date, over 35 identified PCD genes are reported to account for about 70% of screened, well-diagnosed cases [7]. Some PCD gene mutations are never associated with situs abnormalities, connected to a lack of functional requirement for their encoded proteins in the embryonic node [7 8].

It is well established that cilia motility plays a major role in laterality determination, but much remains unknown about the clinical and genetic risk factors for situs defects and CHD pathogenesis in motile ciliopathy disorders [3].

Methods

This is a retrospective cohort study of 389 patients seen in specialist UK clinics with a diagnosis of PCD according to European Respiratory Society (ERS) guidelines [9]. Full details are described in the supplementary methods.

Situs was classified as: (1) situs solitus (SS), defined as normal organ arrangement, (2) situs inversus totalis (SIT), defined as mirror image arrangement of all organs or (3) SA, defined as any abnormal arrangement that was not SS or SIT. A two-stage system was used for organ defect classification (**Table S1)**. Statistical analysis focussed on associations between clinical and genetic factors and two main outcomes: situs abnormality and CHD and/or structural laterality defects. Analysis was performed using Fisher's exact test and univariate and multivariable logistic regression modelling.

Genes were assigned to two groups (A and B) according to whether they have previously been associated to situs abnormalities in the literature (**Table S2):** Group A genes associated with situs abnormalities and Group B genes not previously associated with situs abnormalities.

Results

The clinical data and genetic test results available for analysis in the 389 confirmed PCD patients in the study is shown in supplementary **Figure S1**, along with the details of CHD and laterality defects identified (online supplementary **Table S3**) and full results of statistical regression modelling (online supplementary **Table S4)**.

Situs abnormalities: 49.2% patients had SS, 41.9% had SIT and 8.9% had SA. The distribution of normal and abnormal situs arrangements was assessed for each of 27 PCD genes found to be mutated in the 199 patients for whom both situs was determined and genetics solved. Notably, for 18 genes, patients with bi-allelic mutations had normal or abnormal situs, whilst patients with bi-allelic mutations in the other 9 genes all had normal situs (**Figure 1**). This difference in frequency of situs abnormality between patients with mutations in group B vs group A genes (0/38 vs. 98/161 respectively) highlights a significant association between situs abnormality in our cohort and the literature evidence for situs abnormality (p-value < 0.001, Fisher's exact test) (online supplementary **Table S4,** outcome 1).

Parental consanguinity, ethnicity and functional gene effect were evaluated as potential risk factors for situs abnormality. Only parental consanguinity was found to be significantly associated with situs abnormality (online supplementary **Table S4,** outcome 1). Univariate modelling suggests there is a 77.2% increase in the odds of situs abnormality for patients with consanguineous parents compared to those with non-consanguineous parents (OR = 1.77, p = 0.02, 95% CI (1.09 – 2.88)).

Congenital heart defects and structural laterality defects: 25.2% of patients had CHD and/or laterality defects other than SIT. The prevalence of CHD and/or laterality defects according to situs group is shown in **Figure 2.**

In a risk factor model, only situs abnormality was found to be significantly associated with the presence of CHD and/or laterality defects other than SIT (online supplementary **Table S4**, outcome 2). The univariate model suggests there is an 698% increase in the odds of having CHD and/or structural laterality defects for patients with abnormal situs, compared to the group of patients with normal situs (OR = 7.98, $p < 0.001$, 95% CI (3.57 -17.83)).

Discussion

This is the first study investigating situs and laterality defects in PCD patients from the UK. Compared to previously published studies [5 6], there is a similar situs distribution but we identify at least 3x higher prevalence of CHD in this PCD population (17% of cases). The observed prevalence of laterality defects other than SIT (14.1%) was also high.

The identified prevalence of CHD and laterality defects must be interpreted carefully given the difference in classification systems used to previous studies. We chose to classify according to severity, deciding this was most important for patient care. International consensus on nomenclature and classification for situs and laterality defects would improve comparison between research studies. For completeness, we did also classify our cohort using the same modified Botto et al system [10] as used by previous studies [4-6] (online supplementary **Table S3)**.

The higher observed prevalence amongst our patients to those reported previously could be due to a difference in populations. We have an ethnically diverse cohort, with a high proportion with consanguineous parents, who may have more severe disease phenotypes. A limitation to this study was variation in the availability of detailed imaging data amongst patients. We acknowledge a selection bias is possible for patients with detailed imaging, towards those more likely to have CHD/other laterality defects based on their history or clinical examination.

Given the higher than anticipated prevalence of cardiac and laterality defects identified in this study, we recommend that all patients diagnosed with PCD have a cardiac echocardiogram and abdominal

USS. These are simple, harmless and inexpensive tests. Many of the structural laterality defects are clinically actionable, so are important to detect.

Our study affirms the importance of genetic predisposition to laterality defects in PCD, since a subset of PCD genes were clearly not associated with situs problems.

In summary, this study illustrates that improved knowledge about genotype-phenotype correlations in PCD may facilitate risk predictions for CHD and laterality defects as well as other clinical consequences, allowing for early detection and treatment.

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Author contributions

H.M.M., J.S.C. and C.H. designed the project and are responsible for overall content. S.B. compiled, managed and analysed the clinical and genetic data. S.B., A.S. and B.R. searched clinical records and compiled the clinical data. S.B., M.P.P., M.R.F., S.T., R.P., T.C., J.H. and A.O. performed genetic analyses. A.S., M.D., A.V.R., R.A.H., A.R., S.O., C.J. and P.G. performed clinical cilia functional testing and imaging studies. E.P. advised on and performed statistical analysis. C.O'C., M.R.L., R.W., E.C., P.K., J.S.L., C.H. contributed clinical analysis and data management. V.L.D. and J.S.C. contributed cardiac data management and interpretation. S.B., J.S.C., C.H. and H.M.M. wrote the manuscript. All authors reviewed the data, revised the manuscript for logical content and approved the final version.

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Competing interests

The authors declare they have no competing interests.

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Figure Legends

Figure 1. Situs distribution observed for each PCD gene identified amongst the genetically solved cohort.

This shows the number of patients with normal situs (SS) and abnormal situs (SIT and SA) for each known PCD gene (N=27) amongst the 199 patients identified to have bi-allelic mutations in whom situs was known. No abnormal situs is detected in patients with mutations in nine genes, called group B: *CCDC164, CCDC65, CCNO, HYDIN, MCIDAS, RPGR, RSPH1, RSPH4A* and *RSPH9*.

Figure 2. Distribution of situs arrangements amongst the PCD patients, and a breakdown of CHD and other laterality defects in each situs group.

The number of patients in each category is given. The percentage of patients in each situs group (SS, SIT, SA) was calculated from the total number of patients in whom situs was determined (n=370). The percentage of patients with each category of CHD and/or laterality defect other than SIT was calculated from the total number of patients who fulfilled criteria for organ defect classification (n=234).

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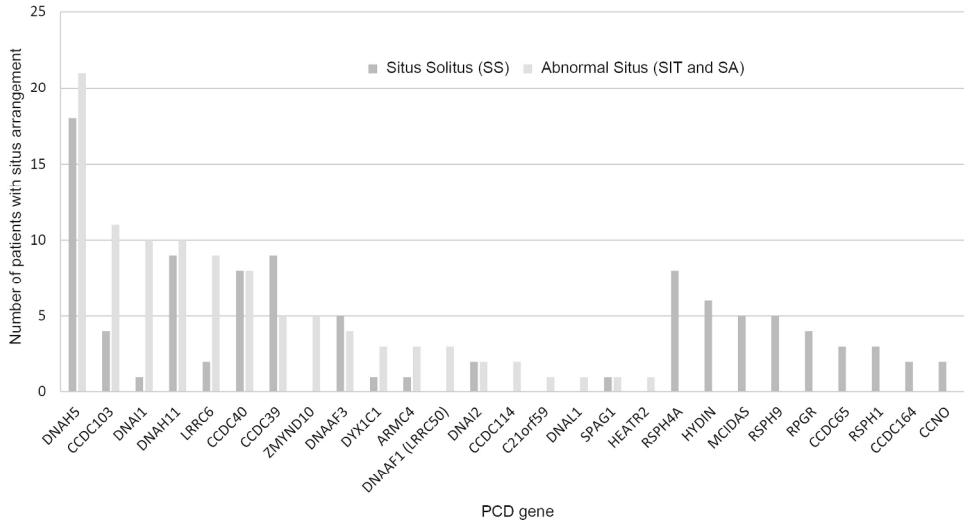

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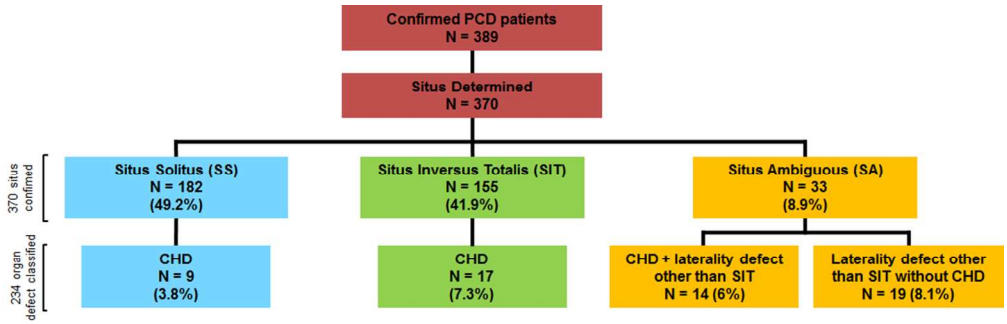

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Sunayna Best BSc MBBS MSc MRCPCH[1,2], Amelia Shoemark BSc PhD[2], Bruna Rubbo MBBS MSc[3,4], Mitali P. Patel BSc MSc[1], Mahmoud R. Fassad MBBCH MSc [1,5], Melissa Dixon BSc PhD[2] Andrew V. Rogers BSc PhD[2], Robert A. Hirst BSc PhD[6], Andrew Rutman CBiol MRSB[6], Sarah Ollosson BSc[2], Claire Jackson BSc MSc PhD[3,4], Patricia Goggin BSc, MMedSci.[3,4], Simon Thomas BSc PhD[7,8], Reuben Pengelly MBiol PhD[7], Thomas Cullup BSc[9], Eleni Pissaridou BSc MSc[10], Jane Hayward BSc PhD[1,9], Alexandros Onoufriadis BSc MSc PhD[11], Christopher O'Callaghan BMedSci BM BS PhD DM[6,12], Michael R. Loebinger MA FRCP PhD[13], Robert Wilson MD FRCP[13], Eddie Chung MBChB MD FRCPCH[10], Priti Kenia MBBS, MD Paediatrics, MRCPCH[14], Victoria L. Doughty BSc PhD[15], Julene S. Carvalho MD PhD FRCPCH[15,16,17], Jane S. Lucas BM PhD [3,4], Hannah M. Mitchison BSc PhD[1*], Claire Hogg MBChB, BSc MRCPCH[2*]

[1]Genetics and Genomic Medicine, University College London, UCL Great Ormond Street Institute of Child Health, London WC1N 1EH, UK

[2] PCD Diagnostic Team, Department of Paediatric Respiratory Medicine, Royal Brompton and Harefield NHS Trust, Sydney Street, London SW3 6NP, UK

[3] Primary Ciliary Dyskinesia Centre, University Hospital Southampton NHS Foundation Trust and Clinical and Experimental Sciences Academic Unit, University of Southampton Faculty of Medicine, Southampton SO17 1BJ, UK

[4] NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, UK

[5] Human Genetics Department, Medical Research Institute, Alexandria University, Alexandria 21561, Egypt

[6] Centre for PCD Diagnosis and Research, Department of Infection, Immunity and Inflammation, RKCSB, University of Leicester, Leicester LE2 7LX, UK

[7] Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton SO17 1BJ, UK

[8] Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury District Hospital, Salisbury SP2 8BJ, UK

[9] North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children, London WC1N 3BH, UK

[10] Population, Policy and Practice Programme, University College London Great Ormond Street Institute of Child Health, 30 Guildford Street, London WC1N 1EH, UK

[11] Department of Medical and Molecular Genetics, Division of Genetics and Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London SE1 9RT, UK

[12] Respiratory, Critical Care & Anaesthesia, UCL Great Ormond Street Institute of Child Health, London WC1N 1EH, UK

[13] Host Defence Unit, Royal Brompton and Harefield NHS Trust, Sydney Street, London SW3 6NP, UK

[14] Department of Respiratory Paediatrics, Birmingham Children's Hospital NHS Foundation Trust, Steelhouse Lane, Birmingham B4 6NH, UK

[15] Brompton Centre for Fetal Cardiology, Royal Brompton and Harefield NHS Trust, Sydney Street, London, SW3 6NP, UK

[16] Fetal Medicine Unit, St George's University Hospitals NHS Foundation Trust, Blackshaw Road, Tooting, London SW17 0QT, UK

[17] Molecular & Clinical Sciences Research Institute, St George's, University of London, Cranmer Terrace, London SW17 0RE, UK

* Shared senior authorship

Supplementary Methods

Included Patients and Clinical Data

This is a retrospective cohort study, designed to investigate the prevalence of situs and visceral defects in UK based patients with PCD and determine whether there are any clinical or genetic risk factors for these. Patients eligible had been diagnosed in a specialist UK PCD clinic according to European Respiratory Society (ERS) guidelines including by transmission electron microscopy (TEM), high speed video microscopy, immunofluorescence and nasal nitric oxide measurement [1 2]; a definite diagnosis defined by a characteristic ciliary ultrastructural abnormality detected by TEM or a bi-allelic mutation in a known PCD gene. Genetic testing was conducted using next generation sequencing and PCD was confirmed where bi-allelic mutations in a known PCD gene with predicted or known pathogenicity in both alleles were identified and confirmed by Sanger sequencing. Paediatric and adult patients were identified from three UK PCD clinical centres (London, Birmingham and Southampton) and participants gave written informed consent to take part. Study recruiters attended the monthly PCD outpatient clinics over the course of a decade with eligible patients seen in the clinic on days of recruitment approached to take part. The protocol was approved by the London Bloomsbury Research Ethics Committee (08/H0713/82). Retrospective clinical data were obtained from electronic records and paper notes, including ethnicity, parental consanguinity, TEM reports and imaging reports. Ethnicity was categorised into three groups: South Asian (Indian, Bangladeshi, Pakistani, Sri Lankan), Caucasian and other (other Asian, Black and mixed ethnicity).

Situs and Organ Defect Classification

Situs classification was performed for all patients in whom the position of at least one thoracic and one abdominal organ was known from chest X-ray and/or other detailed imaging reports. For example, from the chest-X-ray, if the stomach and heart were on the left, the patients were assumed to have SS, and if they were both on the right, they were assumed to have SIT, unless detailed imaging reports were available to provide further clarification. Situs was classified as: (1) situs solitus (SS), defined as normal organ arrangement, (2) situs inversus totalis (SIT), defined as mirror image arrangement of all organs and (3) SA, defined as any abnormal arrangement that was not SS or SIT. The SA group also included cases of apparently isolated dextrocardia or cases with malposition of other organs (e.g. kidney). SS was considered normal situs and SIT and SA were collectively considered abnormal situs.

Organ defect classification was performed on all patients with at least one detailed cardiac (echocardiography, cardiac magnetic resonance imaging (MRI), surgical reports) or abdominal imaging report (abdominal computer tomography (CT), abdominal ultrasound scan (USS), surgical reports). If the patients had undergone surgery, their pre-operative anatomical defect was used for classification. In all cases, available surgical and radiology/echocardiography reports agreed. Only structural congenital abnormalities were included in the classification; acquired abnormalities were not considered. A two-stage classification system was used, as shown in online supplementary **Table S1**. CHD classification was performed first using “CHD present” versus “CHD absent”, modified from previous attempts to classify CHD according to clinical severity [3]. Of note, the “CHD present” category can be further subdivided according to clinical severity into “major” and “simple”. The major CHD category includes those defects classified as “severe” in the International Classification of Disease, ninth revision (ICD-9) [4], as well as abnormalities

which required significant surgical intervention in the first year of life or long term follow up, excluding patent ductus arteriosus (PDA) and isolated septal defects [3 5]. A similar system is used throughout the UK (http://www.ucl.ac.uk/nicor). Patients with CHD also underwent classification according to the modified Botto et al [6] classification of complexity, used in the previous publications of Shapiro et al and Kennedy et al [7-9].

The organ defect classification was performed second, which included two categories: "laterality defect other than SIT present" and "laterality defect other than SIT absent". This system was used to label all visceral and vascular abnormalities detected that were not defined as CHD that potentially resulted from ciliary problems during embryogenesis. Isomerism was classified as a laterality defect other than SIT; if patients with isomerism had associated CHD, this was classified separately [10].

Genetic Analysis

Genetic testing used a variety of gene-mutational analysis performed over a ten-year period: whole exome sequencing was applied in 20% of cases, custom designed ciliopathy gene-panels (TruSeq or Agilent SureSelectXT systems) and a targeted 'clinical exome' (Illumina TruSight One) applied in 70% of cases and first line Sanger sequencing of candidate genes applied in 10% of cases [11]. Families were determined to have "solved" genetic testing when bi-allelic mutations in a known PCD gene with predicted or known pathogenicity in both alleles were identified, then confirmed by Sanger sequencing and where possible by familial segregation analysis. The primary genetic literature references used are contained in **Table S2**.

Statistical Analysis

Statistical analysis focussed on associations between clinical and genetic factors and two main outcomes: situs abnormality and CHD and/or laterality defects other than SIT. Analysis was performed using Fisher's exact test and univariate and multivariable logistic regression modelling. The relative burden of each risk factor was described in odds ratios. The statistical significance level was set to 5%. Data were analysed using Stata Statistical Software (Release 14, College Station, TX: StataCorp LP, 2015).

Figure S1. Summary of clinical data and genetic test results available for analysis in the 389 confirmed PCD patients in the study

Shows the number of patients for whom data was available for situs classification and organ defect classification, as well as the number of patients with genetic test results and known parental consanguinity status. Combinations of data from these categories were used for logistic regression modelling. The four categories are not mutually exclusive, several patients fell into multiple categories. Shaded overlapping areas represent where patients had combinations of data available. The central point shows that 142 patients had data within all 4 categories. Not all categories used for the regression analysis are represented, e.g. ethnicity, and subcategories such as functional gene effect are not shown.

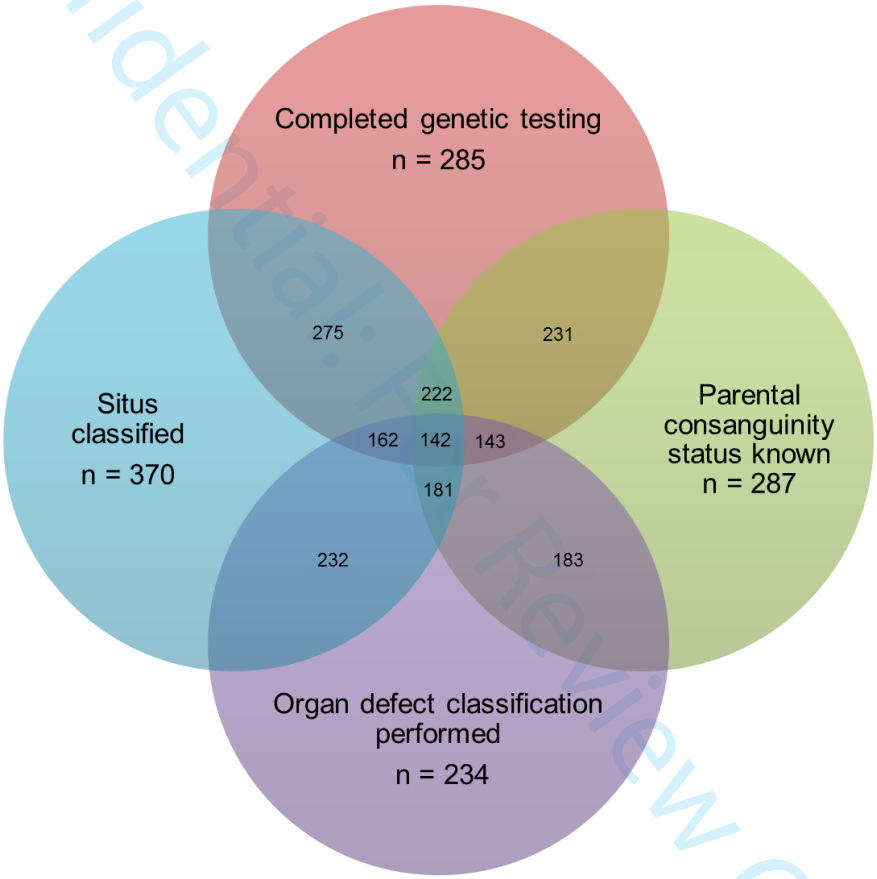

Table S1. Organ defect classification system

Classification Stage	**CHD classification**		**Laterality defect other than SIT classification**	
Categories within classification	**CHD absent**	**CHD present**	**Laterality defect other than SIT absent**	**Laterality defect other than SIT present**
Included Abnormalities	• Normal cardiac anatomy • Abdominal and/or cardiac isomerism without association CHD • Situs inversus without associated CHD	Usually termed simple CHD • ASD • VSD • Isolated valvular stenosis or regurgitation • PDA • Aortopathy Generally termed major CHD • AVSD • TOF • TGA • Truncus arteriosus • Hypoplastic left heart syndrome • Coarctation of the aorta • Tricuspid atresia and other forms of univentricular heart • Pulmonary artery atresia with or without a VSD • Double outlet ventricle • Ebstein's anomaly • Any other CHD requiring significant surgical or catheter intervention in the first year of life, excluding ASD, VSD and PDA	• Normal detailed abdominal imaging report(s) and/or no isomerism detected on cardiac imaging	**Incomplete situs inversus:** • Isolated situs inversus thoracalis • Isolated situs inversus abdominalis **Abdominal visceral abnormalities:** • Intestinal malrotation • Intestinal or biliary atresia • Midline liver • Polysplenia • Asplenia • Structural kidney abnormalities (cystic kidneys, dysplastic kidneys, additional or missing kidneys, malpositioned kidneys) **Vascular abnormalities:** • Abnormalities of major abdominal vessels (e.g. interrupted IVC, duplicated SVC) **Abdominal, thoracic or cardiac isomerism:** • Left isomerism • Right isomerism

CHD = Congenital Heart Disease. ASD = atrial septal defect. VSD = ventricular septal defect. PDA = patent ductus arteriosus. AVSD = atrial-ventricular septal defect. TOF = tetralogy of Fallot. TGA = transposition of the great arteries. IVC = inferior vena cava. SVC = superior vena cava.

Table S2. Genes known to cause PCD

PCD gene	Associated ultrastructural defect	Functional gene effect category	Previously associated with situs abnormalities in the literature?	Reference
CCDC164 (DRC1)	Microtubular disorganisation (MTD)	Involved in the structure and stability of the central pair and nexin links	No (Group B)	12
CCDC65 (DRC2)	MTD			13
GAS8	MTD			14
HYDIN	Normal (subtle central apparatus defect)			15
STK36	Central apparatus defect			16
RPGR	Normal or MTD (syndromic form of PCD)	Photoreceptor connecting cilium protein	No (Group B)	17 18
CCNO	RGMC	Involved in regulation of multiciliated cell differentiation	No (Group B)	19
MCIDAS	RGMC			20
DNAJB13	Central apparatus defect	Encode structural radial spoke proteins	No (Group B)	21
RSPH1	Central apparatus defect			22
RSPH3	Central apparatus defect and MTD			23
RSPH4A	Central apparatus defect			24
RSPH9	Central apparatus defect			24
CCDC39	IDA and MTD	Encode molecular ruler proteins	Yes (Group A)	25 26
CCDC40	IDA and MTD			25 26
ARMC4	ODA defect	Involved in structure and stability of the ODA (encode structural ODA components and factors required for ODA attachment and docking)	Yes (Group A)	27
CCDC114	ODA defect			28
CCDC151	ODA defect			29
DNAH11	Normal (subtle ODA defect)			30
DNAH5	ODA defect			31
DNAI1	ODA defect			32
DNAI2	ODA defect			33
DNAL1	ODA defect			34
TTC25	ODA defect			35
TXNDC3 (NME8)	ODA defect			36
CCDC103	ODA defect			37
C21orf59	IDA and ODA defect	Encode cytoplasmic dynein-arm-assembly machinery proteins	Yes (Group A)	13
DNAAF1 (LRRC50)	IDA and ODA defect			38
DNAAF2 (KTU)	IDA and ODA defect			39
DNAAF3	IDA and ODA defect			40
DNAAF4 (DYX1C1)	IDA and ODA defect			41
DNAAF5 (HEATR2)	IDA and ODA defect			42
LRRC6	IDA and ODA defect			43
PIH1D3	IDA and ODA defect			44
SPAG1	IDA and ODA defect			45
ZYMND10	IDA and ODA defect			46 47

PCD = Primary Ciliary Dyskinesia. MTD = Microtubular Disorganisation. RGMC = Reduced Generation of Multiple Motile Cilia. IDA = Inner Dynein Arm. ODA = Outer Dynein Arm.

Table S3. Features of Primary Ciliary Dyskinesia patients identified to have congenital heart disease (CHD) and/or a structural laterality defect

ID	Detailed imaging reports available	Situs classification*	Cardiac apex position	Position of stomach	Position of liver	Position of spleen	Overall laterality defect (includes SIT)	Laterality defect other than SIT**	Presence of CHD	Botto's CHD classification	Further details of CHD, if available	CHD classification (clinical complexity)
SHN60	CXR, echo, surgical reports	SA	Right	Right	Unknown	Unknown	Abnormal situs; isolated situs inversus thoracalis	Present	Yes	Heterotaxy + CHD	AVSD, pulmonary atresia	Major CHD
SHN32	CXR, echo	SS	Left	Left	Unknown	Unknown	No	Absent	Yes	DORV-TGA	DORV, TGA, coarctation of the aorta, PDA, VSD	Major CHD
RBH66	CXR, echo, abdo USS, CT chest, surgical reports	SIT	Right	Right	Left	Right	Abnormal situs	Absent	Yes	d-TGA	TGA, coarctation of the aorta	Major CHD
SHN92	CXR, echo, surgical reports	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	d-TGA	TGA, VSD	Major CHD
SHN89	CXR, echo, surgical reports	SS	Unknown	Left	Unknown	Unknown	No	Absent	Yes	d-TGA	TGA, pulmonary stenosis, VSD	Major CHD
RBH274	CXR, echo, CT chest, surgical reports	SA	Left	Right	Left	Right	Abnormal situs; Isolated situs inversus abdominalis; Accessory left IVC	Present	Yes	Heterotaxy + CHD	AVSD	Major CHD
RBH149	CXR, echo, abdo USS, surgical reports	SA	Left	Left	Right	Left	Abnormal situs; IVC stenosis	Present	Yes	Heterotaxy + CHD	AVSD, TGA	Major CHD
RBH147	CXR, echo, abdo USS, CT chest, surgical reports	SA	Right	Right	Left	Asplenia	Abnormal situs, right atrial isomerism	Present	Yes	Heterotaxy + CHD	AVSD, Ebstein's anomaly	Major CHD
RBH145	CXR, cardiac MRI, CT chest, surgical reports	SA	Left	Left	Unknown	Unknown	Abnormal situs, left atrial isomerism	Present	Yes	Heterotaxy + CHD	DORV, pulmonary stenosis, VSD, ASD	Major CHD
RBH140	CXR, CT chest, surgical reports	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	DORV	DORV	Major CHD
BCH23	CXR, echo, surgical reports	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	Fallot	TOF	Major CHD
RBH119	CXR, echo, abdo USS, CT chest	SA	Left	Right	Right	Left	Abnormal situs, left atrial isomerism plus Intestinal malrotation	Present	Yes	Heterotaxy + CHD	ASD, bilateral SVC, anomalous IVC drainage (operated 3 weeks of life)	Major CHD
SHN53	Echo, abdo USS	SA	Left	Left	Right	Left	Abnormal situs, left atrial isomerism plus left renal duplication	Present	Yes	Heterotaxy + CHD	Large ASD, multiple small VSDs, coarctation of the aorta	Major CHD
RBH215	CXR, echo, surgical reports	SS	Left	Left	Unknown	Unknown	No	Absent	Yes	d-TGA	TGA	Major CHD
RBH32	CXR, echo, abdo USS, surgical reports	SA	Right	Right	Left	Asplenia	Abnormal situs, right atrial isomerism plus Intestinal malrotation	Present	Yes	Heterotaxy + CHD	Complex cyanotic CHD requiring multiple surgeries in first year of life	Major CHD
RBH170	CXR, echo, abdo USS, CT chest, surgical reports	SA	Left	Right	Left	Right	Abnormal situs	Present	Yes	Heterotaxy + CHD	Complex cyanotic CHD, pulmonary atresia	Major CHD
BCH16	CXR, echo, abdo USS	SA	Left	Right	Left	Right	Abnormal situs; Situs inversus abdominalis, cardiac apex to the left, mirror image bronchial branching pattern	Present	Yes	Heterotaxy + CHD	TGA, pulmonary artery atresia, VSD	Major CHD
RBH169	CXR, echo, abdo USS, CT chest, surgical reports	SIT	Right	Right	Left	Right	Abnormal situs	Absent	Yes	Tricuspid atresia	Tricuspid atresia, VSD, PDA	Major CHD
SHN61	CXR, echo	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	VSD	VSD	Simple CHD
RBH70	CXR, echo, abdo USS	SIT	Right	Right	Left	Right	Abnormal situs	Absent	Yes	ASD 2	ASD	Simple CHD
BCH4	CXR, echo	SS	Left	Left	Unknown	Unknown	No	Absent	Yes	VSD	VSD	Simple CHD

RBH8	CXR, echo, abdo USS	SA	Right	Right	Left	Right	Abnormal situs plus duplex right kidney	Present	Yes	Heterotaxy + CHD	VSD	Simple CHD
RBH63	CXR, echo, abdo USS, CT chest	SS	Left	Left	Right	Left	No	Absent	Yes	VSD	VSD	Simple CHD
RBH55	CXR, echo, abdo USS	SS	Left	Left	Right	Left	No	Absent	Yes	AS	Aortic stenosis and regurgitation	Simple CHD
RBH2	CXR, echo	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	N/A	PDA	Simple CHD
RBH141	CXR, echo	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	VSD	VSD	Simple CHD
RBH79	CXR, echo, abdo USS	SIT	Right	Right	Left	Right	Abnormal situs	Absent	Yes	N/A	PDA	Simple CHD
RBH159	CXR, echo	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	VSD	VSD	Simple CHD
SHN10	CXR, echo	SA	Left	Left	Unknown	Unknown	Abnormal situs, left atrial isomerism	Present	Yes	Heterotaxy + CHD	PDA	Simple CHD
RBH253	CXR, cardiac MRI, CT chest, surgical reports	SA	Left	Left	Unknown	Unknown	Abnormal situs, left atrial isomerism	Present	Yes	Heterotaxy + CHD	Aortic stenosis	Simple CHD
RBH11	CXR, echo	SS	Left	left	Unknown	Unknown	No	Absent	Yes	ASD 2	ASD	Simple CHD
RBH94	CXR, echo, abdo USS	SIT	Right	Right	Left	Right	Abnormal situs	Absent	Yes	N/A	Aortopathy	Simple CHD
RBH101	CXR, echo, abdo USS, CT chest	SIT	Right	Right	Left	Right	Abnormal situs	Absent	Yes	VSD	VSD	Simple CHD
BCH32	CXR, echo	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	VSD	VSD,	Simple CHD
BCH24	CXR, echo, abdo USS	SA	Right	Right	Left	Right	Abnormal situs plus malrotation of SMA/SMV axis	Present	Yes	Heterotaxy + CHD	ASD	Simple CHD
RBH53	CXR, echo, CT chest	SIT	Right	Right	Left	Right	Abnormal situs	Absent	Yes	VSD	VSD	Simple CHD
BCH18	CXR, echo	SIT	Right	Right	Unknown	Unknown	Abnormal situs	Absent	Yes	VSD (?)	Septal defect - no further detail available	Simple CHD
RBH103	CXR, echo, abdo USS, CT chest	SIT	Right	Right	Left	Right	Abnormal situs	Absent	Yes	N/A	Bicuspid aortic valve	Simple CHD
RBH122	CXR, echo, abdo USS, CT chest	SS	Left	Left	Right	Left	No	Absent	Yes	ASD 2	ASD	Simple CHD
BCH9	CXR, echo	SS	Left	Left	Unknown	Unknown	No	Absent	Yes	VSD (?)	Septal defect - no further detail available	Simple CHD
RBH156	CXR, echo, CT chest	SA	Right	Left	Midline	Polysplenia (left)	Abnormal situs; Isolated situs inversus thoracalis; azygos continuation of the IVC	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH13	CXR, echo, abdo USS	SA	Right	Left	Right	Left	Abnormal situs; Isolated situs inversus thoracalis; interrupted IVC with azygos continuation to the SVC	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH105	CXR, echo, abdo USS, CT chest	SA	Right	Left	Right	Left	Abnormal situs; Isolated situs inversus thoracalis	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH64	CXR, echo, abdo USS	SA	Right	Right	Left	Right	Abnormal situs, left atrial isomerism	Present	No CHD	Heterotaxy	No CHD	No CHD
SHN73	CXR, echo	SA	Right	Right	Unknown	Unknown	Abnormal situs, left atrial isomerism	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH286	CXR, echo, CT chest	SA	Right	Left	Unknown	Unknown	Abnormal situs; Isolated situs inversus thoracalis	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH135	CXR, echo, abdo USS, surgical reports	SA	Left	Right	Left	Polysplenia (right)	Abnormal situs plus total jejunal atresia	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH102	CXR, echo, abdo USS	SA	Right	Right	Left	Right	Abnormal situs, left atrial isomerism	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH118	CXR, echo, abdo USS	SA	Left	Right	Left	Right	Abnormal situs, left atrial isomerism	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH27	CXR, echo, abdo USS, CT chest	SA	Left	Right	Left	Polysplenia (right)	Abnormal situs, left atrial isomerism	Present	No CHD	Heterotaxy	No CHD	No CHD
SHN58	CXR, echo	SA	Right	Right	Unknown	Unknown	Abnormal situs, left atrial isomerism	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH153	CXR, echo, abdo	SA	Left	Right	Left	Right	Abnormal situs, left atrial	Present	No CHD	Heterotaxy	No CHD	No CHD

	USS, CT chest						isomerism					
SHN54	CXR, echo, abdo USS	SA	Right	Left	Right	Left	Isolated situs inversus thoracalis, left multicystic dysplastic kidney	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH6	CXR, echo, abdo USS, CT chest	SA	Left	Right	Left	Polysplenia (right)	Abnormal situs; Isolated situs inversus thoracalis	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH81	CXR, echo, abdo USS	SA	Left	Left	Midline	Polysplenia (left)	Abnormal situs	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH15	CXR, echo, abdo USS	SA	Right	Right	Left	Right	Abnormal situs; Azygous vein to left sided SVC	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH90	CXR, echo, abdo USS, CT chest	SA	Left	Right	Central	Right	Abnormal situs, left atrial isomerism plus ileal atresia	Present	No CHD	Heterotaxy	No CHD	No CHD
RBH198	CXR, abdo USS, CT chest	SA	Right	Right	Central	Polysplenia (one spleen in the LUQ and one in RUQ)	Abnormal situs plus polysplenia	Present	Unknown	Heterotaxy (unknown CHD status)	Unknown	Unknown
BCH28	CXR, echo, CT chest	SA	Right	Left	Unknown	Unknown	Abnormal situs, isolated situs inversus thoracalis	Present	No CHD	Heterotaxy	No CHD	No CHD

CXR = Chest X-ray; echo = cardiac echocardiograph; AVSD = Atrial Ventricular Septal Defect; CHD = Congenital Heart Disease; SA = Situs Ambiguous; DORV = Double Outlet Right Ventricle; TGA= Transposition of the Great Arteries; PDA = Patent Ductus Arteriosus; VSD = Ventricular Septal Defect; SS = Situs Solitus; SIT = Situs Inversus Totalis; L/RUQ = left/right upper quadrant; Abdo USS = Abdominal Ultrasound Scan; CT = Computer Tomography; IVC = Inferior Vena Cava; MRI = Magnetic Resonance Imaging; ASD = Atrial Septal Defect; TOF = Tetralogy of Fallot; SVC = Superior Vena Cava; SMA = Superior Mesenteric Artery; SMV = Superior Mesenteric. We note for four CHD cases in this study, that a persistent PDA (RBH2, RBH79, SHN10) or aortopathy (RBH94) could have other aetiologies. *Echocardiographic diagnosis of situs is based on Huhta et al [10]. **Laterality defect other than SIT indicates SA or other possible laterality defect. Botto's Classification of CHD (Level 2) is also shown [6].

Table S4. Results of statistical regression modelling

				Univariate model			Multivariable model		
Risk factor	**Reference category**	**Comparison category**	**Relative frequencies**	**OR**	**p-value**	**95% CI**	**OR**	**p-value**	**95% CI**
OUTCOME 1: SITUS ABNORMALITY									
Parental consanguinity	Non-consanguineous parents	Consanguineous parents	67/119 vs 64/152	1.77	0.02 (significant)	1.09 – 2.88	3.21	0.02 (significant)	1.16 – 8.88
Ethnicity	Caucasian	South Asian	54/92 vs 88/188	1.61	0.06	0.97 – 2.67	0.66	0.48	0.20 – 2.10
		Other	23/51 vs 88/188	0.93	0.83	0.50 - 1.74	0.52	0.27	0.16 – 1.69
Functional gene effect category	Genes involved in structure and stability of the ODA	Genes encoding cytoplasmic dynein arm assembly proteins	26/36 vs 59/95	1.59	0.28	0.69 - 3.67	1.61	0.36	0.58 - 4.49
		Genes encoding ruler proteins	13/30 vs 59/95	0.47	0.07	0.20 - 1.07	0.53	0.21	0.63 - 2.09
Literature evidence for gene association with abnormal situs	Genes thought to be associated with abnormal situs (Group A)	Genes thought to not be associated with abnormal situs (Group B)	0/38 vs 98/161	n/a	n/a	n/a	n/a	n/a	n/a
OUTCOME 2: CHD AND/OR LATERALITY DEFECTS OTHER THAN SIT									
Parental consanguinity	Non-consanguineous parents	Consanguineous parents	23/83 vs 20/100	1.53	0.22	0.77 - 3.05	3.77	0.11	0.75 - 18.95
Ethnicity	Caucasian	South Asian	16/63 vs 29/119	1.06	0.88	0.52 - 2.14	0.36	0.224	0.07 - 1.87
		Other	10/30 vs 29/119	1.55	0.32	0.65 - 3.69	0.43	0.410	0.60 – 3.16
At least one truncating mutation	No truncating mutations	At least one truncating mutation	23/87 vs 6/33	1.62	0.35	0.59 - 4.42	1.75	0.370	0.52- 5.93
Situs abnormality	Normal situs	Abnormal situs	50/126 vs 9/105	7.98	<0.001 (significant)	3.57 - 17.83	8.79	0.002 (significant)	2.28 - 33.89

OR = odds ratio. CI = confidence interval. ODA = outer dynein arm. SIT = situs inversus totalis. Logistic regression modelling was performed with situs abnormality as the dependent dichotomous variable in outcome 1 and the presence of congenital heart disease (CHD) and/or laterality defects other than SIT as the dependent dichotomous variable in outcome 2. For each outcome, separate univariate logistic regression models for each individual risk factor, and one multivariable model incorporating all the risk factors were fitted to the data. Only patients with bi-allelic mutations in known Primary Ciliary Dyskinesia (PCD) genes were included in the tests involving genetic risk factors (functional gene effect category and having at least one truncating mutation). Within the functional gene effect association tests, only the three categories of genes that are thought to be associated with situs abnormalities from the literature were compared (detailed in Table 1). In the multivariate logistic regression, for outcome 1 (situs abnormality) two events (normal/abnormal) were used and 127 subjects included; for outcome 2 (CHD and/or laterality defects other than SIT) two events (presence of defects: no/yes) were used and 101 subjects included.

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