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

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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 system¹⁰ as used by previous studies⁴⁻⁶ (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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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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