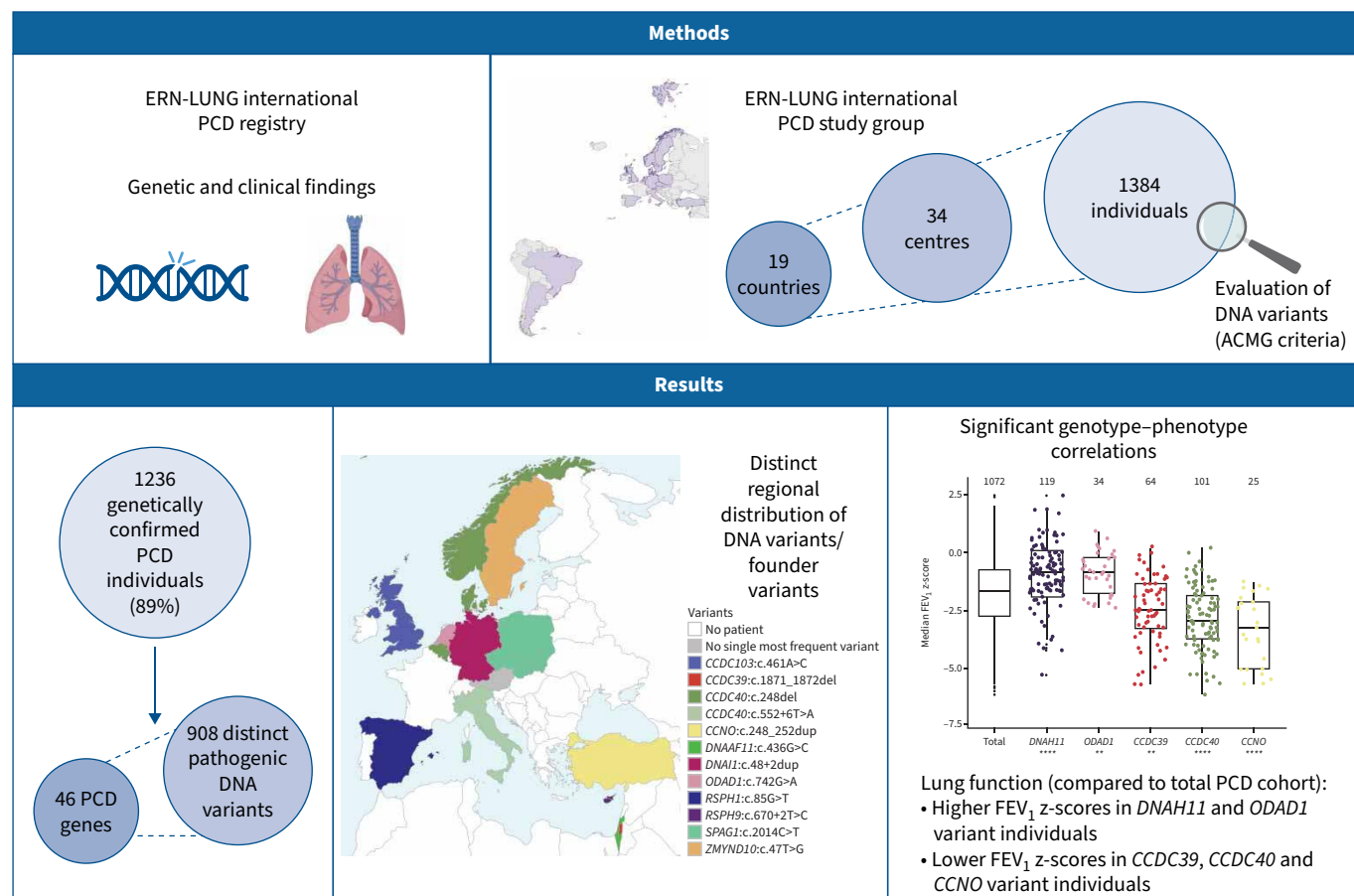


Analyses of 1236 genotyped primary ciliary dyskinesia individuals identify regional clusters of distinct DNA variants and significant genotype–phenotype correlations

Johanna Raidt, Sarah Riepenhausen, Petra Pennekamp, Heike Olbrich, Israel Amirav , Rodrigo A. Athanazio , Micha Aviram, Juan E. Balinotti, Ophir Bar-On , Sebastian F.N. Bode, Mieke Boon, Melissa Borrelli, Siobhan B. Carr , Suzanne Crowley, Eleonora Dehlink, Sandra Diepenhorst, Peter Durdik, Bernd Dworniczak, Nagehan Emiralioglu , Ela Erdem, Rossella Fonnesu, Serena Gracci, Jörg Große-Onnebrink, Karolina Gwozdiewicz, Eric G. Haarman, Christine R. Hansen, Claire Hogg, Mathias G. Holgersen , Eitan Kerem , Robert W. Körner, Karsten Kötz , Panayiotis Kouis , Michael R. Loebinger, Natalie Lorent , Jane S. Lucas , Debora Maj, Marcus A. Mall , June K. Marthin , Vendula Martinu, Henryk Mazurek, Hannah M. Mitchison, Tabea Nöthe-Menchen, Ugur Özçelik, Massimo Pifferi, Andrzej Pogorzelski, Felix C. Ringshausen , Jobst F. Roehmel , Sandra Rovira-Amigo, Nisreen Rumman, Anne Schlegtendal , Amelia Shoemark , Synne Sperstad Kennelly, Ben O. Staar, Sivagurunathan Sutharsan, Simon Thomas, Nicola Ullmann, Julian Varghese, Sandra von Hardenberg, Woolf T. Walker, Martin Wetzke, Michal Witt, Panayiotis Yiallourous, Anna Zschocke, Ewa Ziętkiewicz, Kim G. Nielsen and Heymut Omran



GRAPHICAL ABSTRACT Outline of the study. ERN: European Reference Network; PCD: primary ciliary dyskinesia; ACMG: American College of Medical Genetics and Genomics; FEV₁: forced expiratory volume in 1 s. **: $p \leq 0.01$; ****: $p \leq 0.0001$.



Analyses of 1236 genotyped primary ciliary dyskinesia individuals identify regional clusters of distinct DNA variants and significant genotype–phenotype correlations

Johanna Raidt¹, Sarah Riepenhausen², Petra Pennekamp¹, Heike Olbrich¹, Israel Amirav ^{3,4}, Rodrigo A. Athanazio ⁵, Micha Aviram^{6,7}, Juan E. Balinotti^{8,9}, Ophir Bar-On ^{10,11}, Sebastian F.N. Bode^{12,13}, Mieke Boon¹⁴, Melissa Borrelli¹⁵, Siobhan B. Carr ¹⁶, Suzanne Crowley¹⁷, Eleonora Dehlink¹⁸, Sandra Diepenhorst¹⁹, Peter Durdik²⁰, Bernd Dworniczak¹, Nagehan Emiralioğlu ²¹, Ela Erdem²², Rossella Fonnesu²³, Serena Gracci²³, Jörg Große-Onnebrink¹, Karolina Gwozdziejewicz²⁴, Eric G. Haarman¹⁹, Christine R. Hansen^{25,26}, Claire Hogg¹⁶, Mathias G. Holgersen ²⁷, Eitan Kerem ²⁸, Robert W. Körner²⁹, Karsten Kötz ³⁰, Panayiotis Kouis ³¹, Michael R. Loebinger³², Natalie Lorent ^{33,34}, Jane S. Lucas ^{35,36}, Debora Maj²³, Marcus A. Mall ^{37,38,39}, June K. Marthin ²⁷, Vendula Martinu⁴⁰, Henryk Mazurek²⁴, Hannah M. Mitchison⁴¹, Tabea Nöthe-Menchen¹, Ugur Özçelik²¹, Massimo Pifferi²³, Andrzej Pogorzelski²⁴, Felix C. Ringshausen ^{42,43}, Jobst F. Roehmel ^{37,38,39}, Sandra Rovira-Amigo^{44,45}, Nisreen Rumman^{46,47}, Anne Schlegtendal ⁴⁸, Amelia Shoemark ^{32,49}, Synne Sperstad Kennelly¹⁷, Ben O. Staar^{42,43}, Sivagurunathan Sutharsan⁵⁰, Simon Thomas^{51,52}, Nicola Ullmann⁵³, Julian Varghese², Sandra von Hardenberg⁵⁴, Woolf T. Walker^{35,36}, Martin Wetzke^{43,55,56}, Michal Witt⁵⁷, Panayiotis Yiallourous^{31,58}, Anna Zschocke⁵⁹, Ewa Ziętkiewicz⁵⁷, Kim G. Nielsen^{27,60} and Heymut Omran ¹

¹Department of General Pediatrics, University Hospital Muenster, Muenster, Germany. ²Institute of Medical Informatics, University of Muenster, Muenster, Germany. ³Department of Pediatrics, Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel. ⁴Department of Pediatrics, University of Alberta, Edmonton, AB, Canada. ⁵Pulmonary Division – Heart Institute, Hospital das Clínicas da Faculdade de São Paulo, São Paulo, Brazil. ⁶Pediatric Pulmonary Unit, Soroka Medical Center, Beer Sheva, Israel. ⁷Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel. ⁸Respiratory Center, Ricardo Gutiérrez Children’s Hospital, Buenos Aires, Argentina. ⁹Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina. ¹⁰Pulmonary Institute, Schneider Children’s Medical Center of Israel, Petach-Tikva, Israel. ¹¹Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel. ¹²Center for Pediatrics – Department of General Pediatrics, Adolescent Medicine and Neonatology, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany. ¹³Department of Pediatric and Adolescent Medicine, University Hospital Ulm, Ulm, Germany. ¹⁴Department of Paediatrics, University Hospital, Leuven, Belgium. ¹⁵Department of Translational Medical Sciences, Pediatric Pulmonology, Federico II University, Naples, Italy. ¹⁶Department of Paediatric Respiratory Medicine and Primary Ciliary Dyskinesia Centre, Royal Brompton Hospital and National Heart and Lung Institute, Imperial College London, London, UK. ¹⁷Paediatric Department of Allergy and Lung Diseases, Oslo University Hospital, Oslo, Norway. ¹⁸Division of Pediatric Pulmonology, Allergy and Endocrinology, Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria. ¹⁹Department of Pediatric Respiratory Medicine and Allergy, Emma Children’s Hospital, Amsterdam University Medical Centers, Amsterdam, The Netherlands. ²⁰Department of Pediatrics, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia. ²¹Division of Pediatric Pulmonology, Faculty of Medicine, Hacettepe University, Ankara, Turkey. ²²Department of Pediatric Pulmonology, Marmara University School of Medicine, Istanbul, Turkey. ²³Department of Paediatrics, University Hospital of Pisa, Pisa, Italy. ²⁴Department of Pneumology and Cystic Fibrosis, Institute of Tuberculosis and Lung Diseases, Rabka, Poland. ²⁵Department of Pediatrics, Institution of Clinical Sciences, Lund University, Lund, Sweden. ²⁶Section for Lung Medicine, Metabolism and Neurology, Pediatrics Clinic, Skane University Hospital, Lund, Sweden. ²⁷Danish Primary Ciliary Dyskinesia Centre, Paediatric Pulmonary Service, Department of Paediatrics and Adolescent Medicine, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark. ²⁸Department of Pediatrics and Pediatric Pulmonology, Hadassah Hebrew University Medical Center, Jerusalem, Israel. ²⁹Department of Pediatrics, Faculty of Medicine and University Hospital, University of Cologne, Cologne, Germany. ³⁰Department of Pediatrics, Queen Silvias Children Hospital, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden. ³¹Respiratory Physiology Laboratory, Medical School, University of Cyprus, Nicosia, Cyprus. ³²Royal Brompton and Harefield Hospitals and National Heart and Lung Institute, Imperial College London, London, UK. ³³Department of Respiratory Diseases, University Hospitals Leuven, Leuven, Belgium. ³⁴Department Chrometa, BREATHE Laboratory, Katholieke Universiteit Leuven, Leuven, Belgium. ³⁵Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, UK. ³⁶Primary Ciliary Dyskinesia Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK. ³⁷Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany. ³⁸German Center for Lung Research (DZL), associated partner site, Berlin, Germany. ³⁹Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany. ⁴⁰Department of Paediatrics, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic. ⁴¹Genetics and Genomic Medicine Department, University College London, UCL Great Ormond Street Institute of Child Health, London, UK. ⁴²Department of Respiratory Medicine, Hannover Medical School (MHH),

Hannover, Germany. ⁴³Biomedical Research in End-Stage and Obstructive Lung Disease Hannover (BREATH), German Center for Lung Research (DZL), Hannover, Germany. ⁴⁴Paediatric Pulmonology Section, Department of Paediatrics, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Universitat Autònoma de Barcelona, Barcelona, Spain. ⁴⁵Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain. ⁴⁶Department of Pediatrics, Faculty of Medicine, Makassed Hospital, Al-Quds University, East Jerusalem, Palestine. ⁴⁷Section of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA. ⁴⁸University Children's Hospital, Ruhr University Bochum, Katholisches Klinikum Bochum, Bochum, Germany. ⁴⁹Division of Molecular and Clinical Medicine, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK. ⁵⁰Department of Pulmonary Medicine, Adult Cystic Fibrosis Center, University Hospital Essen – Ruhrlandklinik, University of Duisburg-Essen, Essen, Germany. ⁵¹Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury, UK. ⁵²Human Genetics and Genomic Medicine, University of Southampton Faculty of Medicine, Southampton, UK. ⁵³Pneumology and Cystic Fibrosis Unit, Academic Department of Pediatrics, Bambino Gesù Children's Hospital, Rome, Italy. ⁵⁴Department of Human Genetics, Hannover Medical School, Hannover, Germany. ⁵⁵Department of Paediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany. ⁵⁶Airway Research Center North (ARCN) Lübeck, German Center for Lung Research (DZL), Lübeck, Germany. ⁵⁷Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland. ⁵⁸Pediatric Pulmonology Unit, Hospital "Archbishop Makarios III", Nicosia, Cyprus. ⁵⁹Department of Pediatric and Adolescent Medicine, Pediatrics III, Medical University, Innsbruck, Austria. ⁶⁰Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark.

Corresponding author: Heymut Omran (Heymut.Omran@ukmuenster.de)



Shareable abstract (@ERSpublications)

The distribution of affected PCD genes and pathogenic gene variants differs markedly within Europe and beyond due to several founder variants. The PCD genotype can predict diagnostic and phenotypic features such as the course of lung function. <https://bit.ly/44AbHTY>

Cite this article as: Raidt J, Riepenhausen S, Pennekamp P, *et al.* Analyses of 1236 genotyped primary ciliary dyskinesia individuals identify regional clusters of distinct DNA variants and significant genotype–phenotype correlations. *Eur Respir J* 2024; 64: 2301769 [DOI: 10.1183/13993003.01769-2023].

Copyright ©The authors 2024.

This version is distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0. For commercial reproduction rights and permissions contact permissions@ersnet.org

This article has an editorial commentary:
<https://doi.org/10.1183/13993003.01026-2024>

Received: 17 Oct 2023
Accepted: 3 May 2024

Abstract

Background Primary ciliary dyskinesia (PCD) represents a group of rare hereditary disorders characterised by deficient ciliary airway clearance that can be associated with laterality defects. We aimed to describe the underlying gene defects, geographical differences in genotypes and their relationship to diagnostic findings and clinical phenotypes.

Methods Genetic variants and clinical findings (age, sex, body mass index, laterality defects, forced expiratory volume in 1 s (FEV₁)) were collected from 19 countries using the European Reference Network's ERN-LUNG international PCD Registry. Genetic data were evaluated according to American College of Medical Genetics and Genomics guidelines. We assessed regional distribution of implicated genes and genetic variants as well as genotype correlations with laterality defects and FEV₁.

Results The study included 1236 individuals carrying 908 distinct pathogenic DNA variants in 46 PCD genes. We found considerable variation in the distribution of PCD genotypes across countries due to the presence of distinct founder variants. The prevalence of PCD genotypes associated with pathognomonic ultrastructural defects (mean 72%, range 47–100%) and laterality defects (mean 42%, range 28–69%) varied widely among countries. The prevalence of laterality defects was significantly lower in PCD individuals without pathognomonic ciliary ultrastructure defects (18%). The PCD cohort had a reduced median FEV₁ z-score (−1.66). Median FEV₁ z-scores were significantly lower in *CCNO* (−3.26), *CCDC39* (−2.49) and *CCDC40* (−2.96) variant groups, while the FEV₁ z-score reductions were significantly milder in *DNAH11* (−0.83) and *ODAD1* (−0.85) variant groups compared to the whole PCD cohort.

Conclusion This unprecedented multinational dataset of DNA variants and information on their distribution across countries facilitates interpretation of the genetic epidemiology of PCD and indicates that the genetic variant can predict diagnostic and phenotypic features such as the course of lung function.

Introduction

Primary ciliary dyskinesia (PCD) (Mendelian Inheritance in Man (MIM) 244400) represents a group of rare genetic disorders characterised by impaired function, structure or generation of multiple motile cilia on epithelial cells lining the airways. Impaired mucociliary clearance leads to chronic mucopurulent airway disease that progresses to irreversible lung damage. Dysfunctional motile cilia present in other tissues can result in non-respiratory disease manifestations such as infertility, laterality defects or, less commonly, hydrocephalus [1]. PCD demonstrates a considerable phenotypic and genetic heterogeneity that often hampers diagnosis. The estimated prevalence ranges from one in 4000 to one in 20 000 and, so far, more than 50 genes have been described to be involved in PCD [1–6]. Notably, data on the regional prevalence



of PCD genotypes across Europe are limited to a handful of country-specific studies [4, 6–10]. Therefore, we analysed the regional prevalence of PCD genotypes across countries using data compiled by the European Reference Networks' ERN-LUNG network (<https://ern-lung.eu>). The ERN-LUNG international PCD Registry systematically collects data from PCD individuals such as diagnostic results, natural history, incidence, clinical presentation, treatment and course of disease [11–13]. We assembled data for 19 different countries across Europe, Asia and South America to explore the global impact of genotypes on clinical aspects of the disease, including lung function. In this largest multinational cohort to date of genetically diagnosed PCD individuals, we revealed marked regional differences of PCD genotypes and identified substantial genotype–phenotype correlations.

Methods

Summary of applied methods

Please see the supplementary material for a detailed method section.

The study used data from previously genotyped individuals of the ERN-LUNG international PCD Registry. Genetic variants were evaluated according to American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) guidelines [14]. Only pathogenic variants were included for further analyses. Several clinical parameters, such as age, sex, body mass index (BMI), laterality status and forced expiratory volume in 1 s (FEV₁), were evaluated. Groups were categorised according to genotypes associated with different ciliary ultrastructural phenotypes. Statistical analysis was performed using R (www.r-project.org), with adjustments for multiple comparisons.

Results

Study population

In this study, 34 centres from 19 countries participated: 15 from Europe, two from Asia (Israel, Palestine) and two from South America (Brazil, Argentina). The number of included PCD individuals differed between centres, ranging from three to 190 (median 25, interquartile range (IQR) 10–38.5), and between countries, ranging from three to 321 (median 32, IQR 17.5–92). Following independent evaluation at the coordinating centre, 148 among 1384 individuals submitted to the study (11%, 0–36% per country) did not have a confirmed genetic diagnosis according to ACMG/AMP guidelines [14]. The most frequent reasons for genetically unconfirmed diagnoses were 1) a single variant in a PCD gene without a second variant identified (in cases of autosomal-recessive inheritance); 2) two heterozygous variants in two different PCD genes (in cases of autosomal-recessive inheritance); 3) genetic variants in candidate but not in known PCD genes; 4) bi-allelic yet not reported genetic variants of unknown significance (class 3) without a consistent clinical phenotype or further confirmatory diagnostic findings such as transmission electron microscopy (TEM), immunofluorescence microscopy analyses or high-speed videomicroscopy; or 5) benign/likely benign (class 1/class 2) variants in known PCD genes. The remaining 1236 individuals with confirmed genetic diagnoses were included in further analyses. The median age of the study population was 21.6 years (IQR 15.4–32.2 years, as of January 2023), 428 individuals (35%) were <18 years old and 808 (65%) were >18 years old. Data on age at diagnosis were available for 947 individuals, showing a median of 10 years for age at diagnosis (IQR 4.4–17 years, range 0–77.7 years). The median age at diagnosis for participants with laterality defects was 8 years (IQR 1.08–16.3 years) compared to 11 years for participants without laterality defects (IQR 6–17.9 years) ($p < 0.0001$).

A total of 615 individuals were male (50%) and 621 were female (50%) (supplementary figure E1). The median BMI of the cohort was $20.3 \text{ kg} \cdot \text{m}^{-2}$ (IQR $17.4\text{--}23.8 \text{ kg} \cdot \text{m}^{-2}$) with a median BMI z-score for individuals <19 years old of 0.00 (IQR $-0.7\text{--}0.07$, $n=652$). The median BMI for individuals >19 years old was $22.7 \text{ kg} \cdot \text{m}^{-2}$ (IQR $20.4\text{--}25.6 \text{ kg} \cdot \text{m}^{-2}$, $n=441$). There were no significant differences in median BMI and age between the gene groups (supplementary table E1).

Genotypes in PCD individuals

Overall, 908 distinct disease-causing variants in 46 PCD-associated genes were detected in the group of 1236 PCD individuals (supplementary table E2), of whom 687 (56%) had homozygous and 528 (43%) had compound heterozygous DNA variants. Only 20 individuals (2%) had hemizygous, X-linked variants (*OFD1*, *DNAAF6* and *RPGR*), while one individual carried an autosomal dominant variant (*FOXJ1*). The majority of allele frequencies (99.7%) for the genetic variants of the study were <0.005 , according to the Genome Aggregation Database (gnomAD) for European (non-Finnish) ancestry (supplementary table E2). The most frequently affected genes, in individuals with bi-allelic pathogenic variants, were *DNAH5* ($n=275$, 22%), *DNAH11* ($n=142$, 11%), *CCDC40* ($n=115$, 9%), *DNAI1* ($n=74$, 6%), *CCDC39* ($n=66$, 5%) and *SPAG1* ($n=51$, 4%) (figure 1).

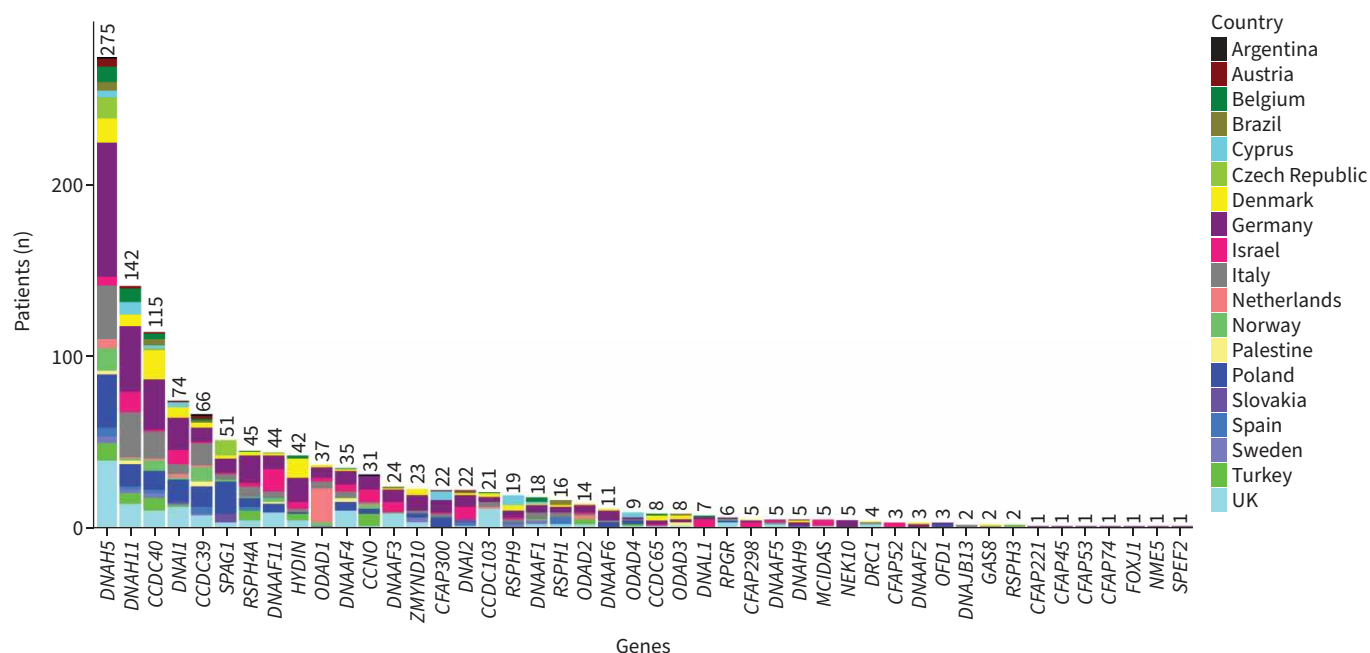


FIGURE 1 The regional distribution and number of individuals with confirmed pathogenic variants in primary ciliary dyskinesia-associated genes among 34 centres from 19 different countries.

Regional distribution of the mutated PCD genes and pathogenic variants

The spectrum of mutated PCD-associated genes differed markedly across the 19 countries. *DNAH5* was overall the most frequently affected gene, both in the whole study cohort (figure 1) and in 12 of 19 participating countries (63%). The most common *DNAH5* variant, c.10815del, was primarily observed in Northern and Central Europe ($\times 46$; figure 2a). The regional distribution of the most frequent pathogenic variants in other selected PCD genes (*CCDC40*, *DNAI1*, *SPAG1*, *CCNO*) is illustrated in figure 2b–e. The *CCDC40* variant c.248del ($\times 69$) was frequently observed in the northern and central parts of Europe. The *DNAI1* variant c.48+2dup ($\times 60$) was prevalent in Northern and Central Europe. Interestingly, the *SPAG1* variant c.2014C>T ($\times 56$) showed a high frequency in the Slavic region including Poland, Czech Republic and Slovakia. The *CCNO* variant c.248_252dup ($\times 18$) was mainly present in Turkey, whereas the *CCNO* variant c.258_262dup ($\times 16$) was mainly present in Israel. Analysis of the most frequent variants per country provided another perspective for PCD-associated genetic diversity. Interestingly, in spite of the overall high involvement of *DNAH5*, none of its variants was reported as the most frequent in any of the analysed countries. The most frequent variants per country were found in 11 different PCD genes (figure 3). More data on the distribution of frequent variants in PCD-associated genes per country can be found in supplementary figure E2.

Genotype–phenotype correlations

Distribution of predicted ultrastructural ciliary phenotypes

Based on the genotypes, we assessed the proportion of patients who could have been successfully diagnosed by TEM [5, 15]. In total, 894 individuals (72%) had DNA variants associated with pathognomonic ciliary ultrastructure defects detectable by TEM (class I defects). The remaining 342 individuals (28%) had DNA variants not associated with hallmark pathognomonic ciliary ultrastructure defects. The proportion of PCD individuals with genetic variants associated with hallmark pathognomonic ciliary ultrastructure defects differed significantly among countries, ranging from 47% to 100% (supplementary figure E3).

Laterality defects

Information on the laterality status was available for 1195 of 1236 individuals (97%). 676 PCD individuals (55%) had normal body composition (situs solitus). 519 individuals were reported to have laterality defects (42%) (figure 4), of whom 482 had situs inversus totalis (39%) and 37 had situs ambiguus (3%). Laterality defects were present in individuals with DNA variants in both the genes associated with hallmark pathognomonic ciliary ultrastructure defects (*CCDC103*, *ODAD1*, *ODAD2*, *ODAD3*, *ODAD4*,

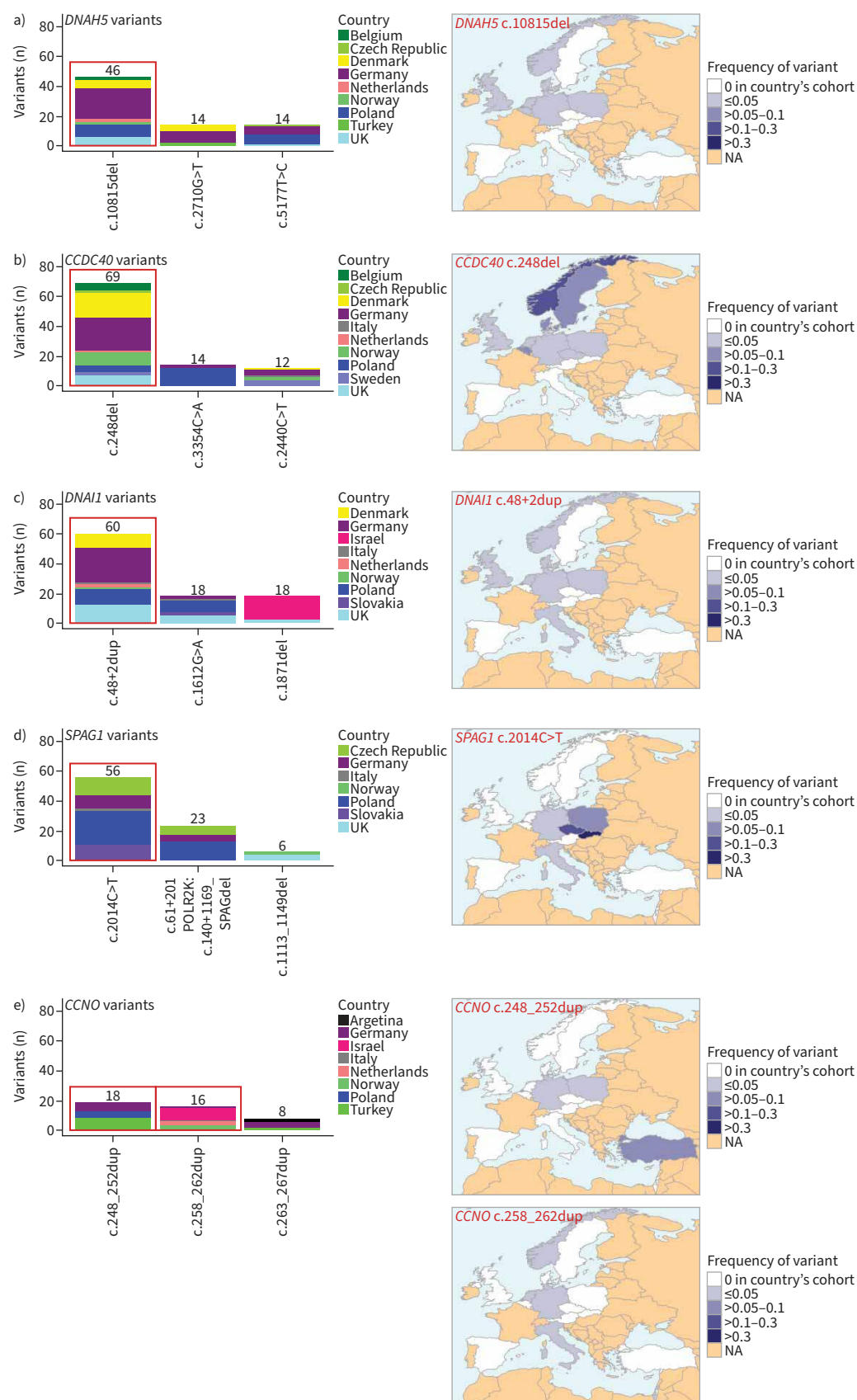


FIGURE 2 The most frequent pathogenic genetic variants in selected primary ciliary dyskinesia genes and their regional distribution. The most common variants in *DNAH5*, *CCDC40*, *DNAI1*, *SPAG1* and *CCNO* show regional

clusters. a) The most common *DNAH5* variant c.10815del (×46) is prevalent in northern Europe. b) The most common *CCDC40* variant c.248del (×69) is also frequently reported in the northern parts of Europe. c) The most common *DNAI1* variant c.48+2dup (×60) predominantly occurs in northern Europe and neighbouring countries. d) The most common *SPAG1* variant c.2014C>T (×56) shows a dominant regional distribution in the Slavic countries Poland, Czech Republic and Slovakia. e) In *CCNO*, there are two frequent genetic variants: c.248_252dup (×18) mainly occurs in Turkey, whereas c.258_262dup (×16) is mainly reported in Israel. NA: not available.

DNAH5, *DNAH9*, *DNAI1*, *DNAI2*, *DNAL1*, *DNAAF1*, *DNAAF2*, *DNAAF3*, *DNAAF4*, *DNAAF5*, *DNAAF6*, *DNAAF11*, *CFAP298*, *CFAP300*, *SPAG1*, *ZMYND10*, *CCDC39* and *CCDC40*) and in the genes *DNAH11*, *FOXJ1*, *CFAP45*, *CFAP52*, *CFAP53* and *OFD1*, which are not associated with pathognomonic ciliary ultrastructure defects. No laterality defects were present in individuals with DNA variants in the genes

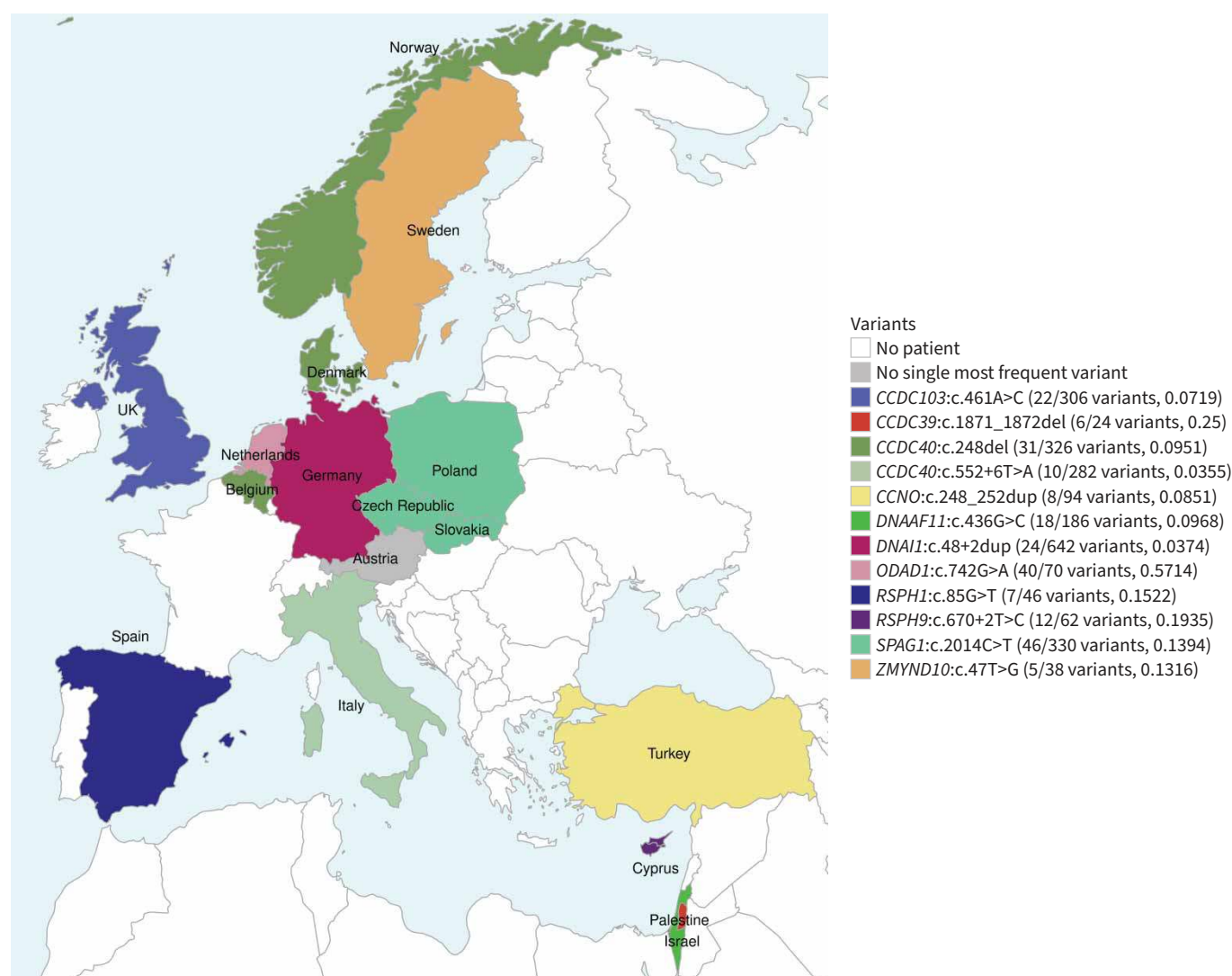


FIGURE 3 The most frequent pathogenic gene variants associated with primary ciliary dyskinesia per country. There are clear regional differences between countries. The total absolute and relative frequency of the most frequent variant per country is shown in brackets. The variant c.2014C>T in *SPAG1* (mint green) is the most frequently reported variant in Poland (24 out of 268 variants, 0.0896), the Czech Republic (12 out of 46 variants, 0.2609) and Slovakia (10 out of 16 variants, 0.625). The variant c.742G>A in *ODAD1* (pale pink) prevails in the Netherlands. The variant c.248_252dup in *CCNO* (yellow) is the most frequently detected variant in Turkey and c.248del in *CCDC40* (dark green) is the most frequently detected variant in Denmark (17 out of 182 variants, 0.0934), Norway (9 out of 80 variants, 0.1125) and Belgium (5 out of 64 variants, 0.0781). Despite the overall high involvement of *DNAH5* (figure 1), none of its variants was identified as the most frequent in any of the countries. Argentina and Brazil are not shown (no most frequent genetic variant).

HYDIN, *SPEF2*, *CFAP221*, *CFAP74*, *RSPH1*, *RSPH3*, *RSPH4A*, *RSPH9*, *DNAJB13*, *NME5*, *GAS8*, *DRC1*, *CCDC65*, *RPGR*, *CCNO*, *MCIDAS* and *NEK10*. The overall prevalence of laterality defects was significantly higher in the group of PCD individuals with genetic variants associated with hallmark pathognomonic ultrastructure defects than in the rest of the cohort (51% versus 18%; $p < 0.0001$) (figure 4). The regional distribution of laterality defects varied widely among the participating countries with the lowest prevalence in Turkey (28%), the Netherlands (31%), Germany (37%), Spain (39%) and Israel (40%) (figure 4).

Lung function

Lung function data were available for 1072 genotyped individuals, with 10 022 FEV₁ values in total. The median number of FEV₁ z-scores of the participants was 4 (IQR 2–8, range 1–268). 948 individuals had more than one FEV₁ measurement; 833 individuals had measurements in a period of more than 1 year, with a median time period of 3.8 years (IQR 2.2–7.9 years, range 1–40.9 years). The median FEV₁ z-score was –1.66 (IQR –2.75– –0.752) for the whole study cohort (figure 5a), with progressively lower FEV₁ z-scores in the groups of older individuals (figure 5b). 528 PCD subjects had FEV₁ data represented in more than one age bin. Individuals with laterality defects had a median FEV₁ z-score of –1.65 (IQR –2.69– –0.753) compared to the median FEV₁ z-score of –1.67 for individuals without laterality defects (IQR –2.81– –0.779) (nonsignificant, $p > 0.05$). However, we found that distinct gene defects were associated with either a more severe or a more subtle loss of lung function (figure 5, supplementary figure E4). The group of individuals with *CCNO* variants ($n=25$) showed the poorest median FEV₁ z-score, which was significantly lower than the rest of the cohort (–3.26, IQR –5.04– –2.13, $p < 0.0001$; figure 5a, b; supplementary figure E4). Genetic defects in *CCDC39* ($n=64$) and *CCDC40* ($n=101$) also resulted in significantly lower median FEV₁ z-scores compared to the rest of the cohort (*CCDC39*: –2.49, IQR

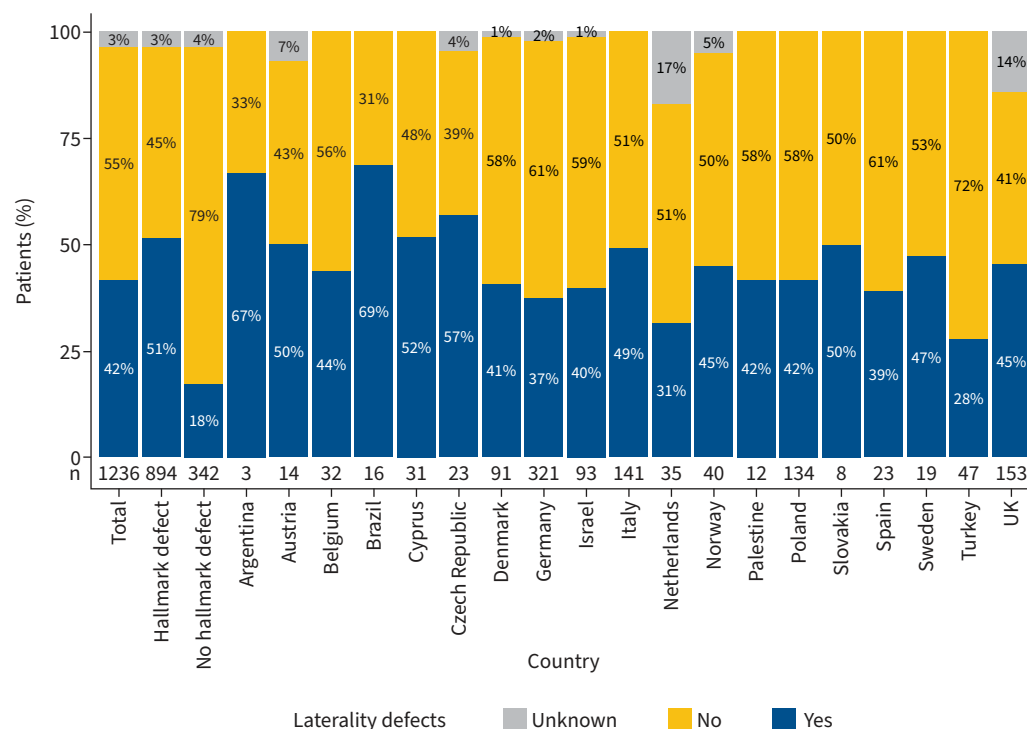


FIGURE 4 Prevalence of laterality defects per predicted ciliary ultrastructure and country. The prevalence of laterality defects is 42% in the total study cohort ($n=519$ individuals with laterality defects). There is a significant difference between the groups stratified according to the predicted effect of genetic variants on ciliary ultrastructure. In the group of 894 individuals with genetic variants associated with pathognomonic ciliary ultrastructure defects detectable by transmission electron microscopy, 51% of individuals ($n=457$) have laterality defects. In contrast, in the group of individuals with genetic variants not associated with defective ciliary ultrastructure hallmark, only 18% of individuals ($n=55$) have laterality defects ($p < 0.0001$). The prevalence of laterality defects varies widely among the participating countries and ranges from 28% to 69%. It is lowest in Turkey (28%), the Netherlands (31%), Germany (37%), Spain (39%) and Israel (40%).

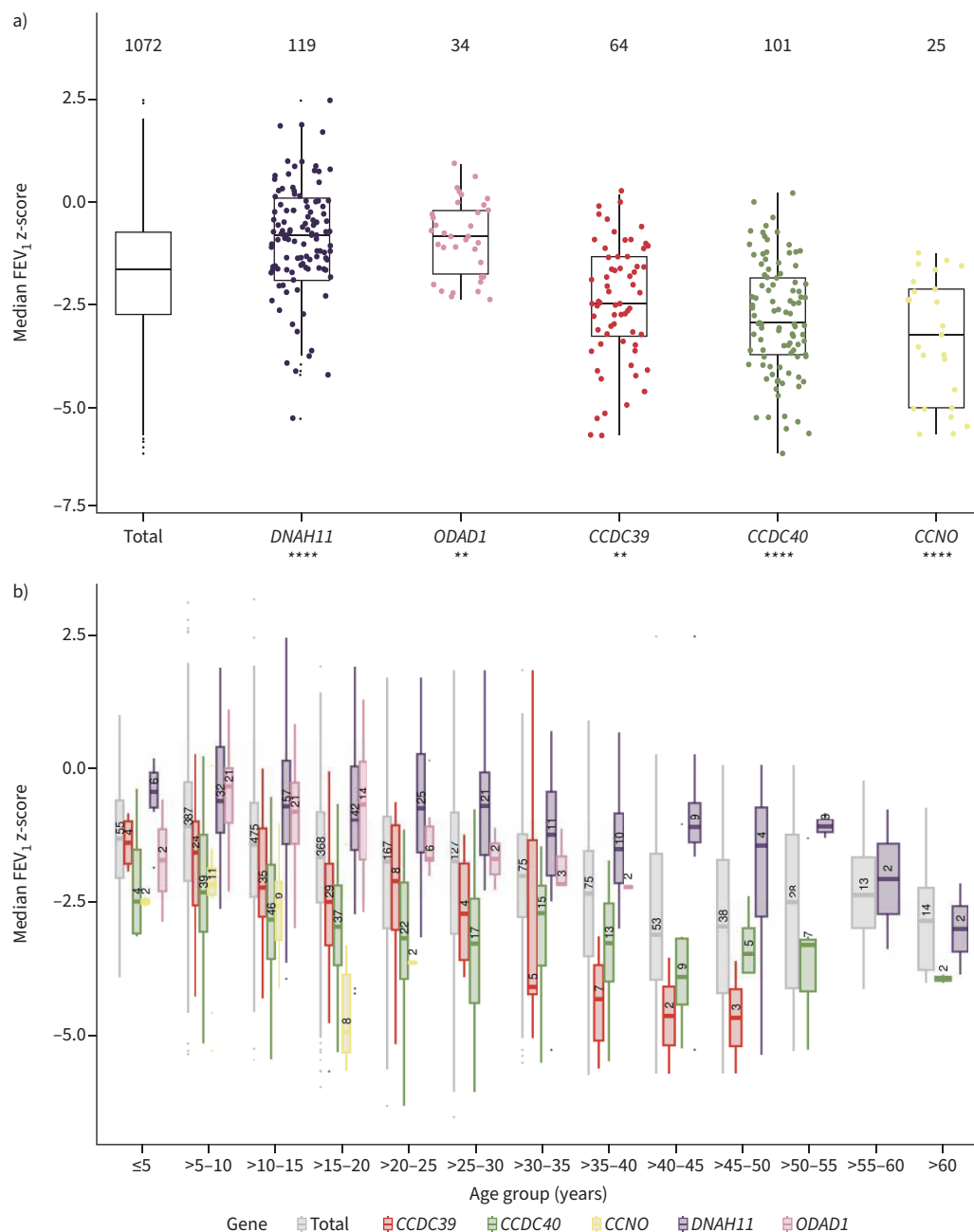


FIGURE 5 Median forced expiratory volume in 1 s (FEV₁) z-scores of the whole primary ciliary dyskinesia (PCD) cohort and distinct PCD groups. **a)** The median FEV₁ z-score of the overall PCD cohort is -1.66 (interquartile range (IQR) -2.75 – -0.752). Individuals with *CCNO* variants ($n=25$) show a significantly lower median FEV₁ z-score (-3.26 , IQR -5.04 – -2.13 , $p<0.0001$) compared to the rest of the cohort. Individuals with DNA variants in *CCDC39* ($n=64$) and *CCDC40* ($n=101$) associated with microtubular disorganisation and inner dynein arm defects exhibit median FEV₁ z-scores significantly lower than the rest of the cohort (*CCDC39*: -2.49 , IQR -3.28 – -1.37 , $p<0.01$; *CCDC40*: -2.96 , IQR -3.77 – -1.86 , $p<0.00001$). The group of individuals with *DNAH11* ($n=119$) and *ODAD1* variants ($n=34$) show significantly higher median FEV₁ z-scores compared to the rest of the cohort (*DNAH11*: -0.831 , IQR -1.57 – -0.0984 , $p<0.0001$; *ODAD1* -0.850 , IQR -1.57 – -0.0984 , $p<0.01$). Significant differences between distinct gene groups and the rest of the cohort are marked with asterisks. $p\leq 0.05$ was considered significant. **: $p\leq 0.01$; ****: $p\leq 0.0001$. **b)** The study cohort was divided into consecutive 5-year age groups to analyse age-dependence of FEV₁ z-scores. 528 PCD individuals have FEV₁ data represented in more than one age bin. Groups of older PCD individuals have increasingly lower FEV₁ z-scores (grey bars). The groups of individuals with *CCNO*, *CCDC39* and *CCDC40* variants have lower FEV₁ z-scores, while individuals with

DNAH11 and *ODAD1* variants have higher FEV₁ z-scores in most age bins compared to the total cohort. However, the median FEV₁ z-scores of the *DNAH11* and *ODAD1* variant group of individuals aged >60 or >30–35 years, respectively, show similarly low values as the total PCD cohort.

–3.28– –1.37, $p < 0.01$; *CCDC40*: –2.96, IQR –3.77– 1.86, $p < 0.00001$), and showed lower values over the entire age range (figure 5a, b; supplementary figure E4). In contrast, the subgroups of individuals with variants in *DNAH11* ($n=119$) and in *ODAD1* ($n=34$) had significantly higher median FEV₁ z-scores compared to the rest of the cohort (*DNAH11*: –0.83, IQR –1.57– –0.098, $p < 0.0001$; *ODAD1*: –0.85, IQR –1.80– –0.15, $p < 0.01$; figure 5a, b). Detailed information regarding median FEV₁ z-scores and FEV₁ % predicted for all gene groups is provided in supplementary table E1.

Discussion

In this multinational study, the genetic diagnosis was confirmed in 1236 individuals (89%), who harboured 908 different disease-causing genetic variants in 46 different PCD genes, confirming the high degree of genetic heterogeneity in PCD (figure 1). In the whole study cohort, *DNAH5* was the most frequently implicated gene, consistent with previous reports [8, 16, 17]. Our study revealed marked regional differences in this distribution within and beyond Europe (figures 1–3), suggesting the presence of several different founder variants. It is known that the presence of founder variants results in a highly variable prevalence of monogenic diseases in Europe and other parts of the world, *e.g.* F508del in the *CFTR* gene responsible for cystic fibrosis [18, 19]. This is also true for PCD, but much more complex because of the high degree of genetic heterogeneity. Our findings are consistent with previous studies that have reported recurrent gene variants, including *DNAH5*:c.10815del [17], *DNAI1*:c.48+2dup [20], *CCDC40*:c.248del [21], *ODAD1*:c.742G>A [22, 23], *SPAG1*:c.2014C>T [24, 25], *CCDC39*:c.1871_1872del [10], *CCDC103*:c.461A>C [26, 27], *HYDIN*:c.922A>T [28], *CFAP300*:c.198_200del [29, 30], *CCNO*:c.258_262dup [31], *MCIDAS*:c.1142G>A [32, 33], *DNAL1*:c.449A>G [34], *RSPH9*:c.670+2T>C [9], *CFAP300*:c.98_106del [35], *CCDC40*:c.552+6T>A [36], *RSPH4A*:c.1391G>A [37, 38] and *ZMYND10*:c.47T>G [39]. In our study, the most pronounced regional cluster was seen for the founder variant c.2014C>T in *SPAG1*, which prevailed in the Slavic countries Poland, Czech Republic and Slovakia. We also found regional clustering of other founder variants (supplementary figure E2).

Recently, HANNAH *et al.* [16] estimated the global prevalence of PCD and predicted the most frequent pathogenic genetic variants and genes associated with PCD for different ethnicities, using the Hardy–Weinberg calculation of the prevalence of bi-allelic variants based on publicly available allele frequencies in large genome sequence databases. In our study, the most frequently affected genes were *DNAH5*, *DNAH11*, *CCDC40*, *DNAI1* and *CCDC39*, consistent with the prediction. However, the order of the affected genes and detection of certain alleles differed slightly. For example, variants predicted to be present in the Ashkenazi population were detected in our Israeli PCD group (*CFAP298*:c.735C>G; *CCNO*:c.638T>C; *DNAI1*:c.1490G>A), but other frequent alleles were not detected at all (*e.g.* *DNAAF1*:c.1698+1G>A; *ZMYND10*:c.599+1G>A). Therefore, predictions based on available allele frequencies from large sequence databases are helpful, but real patient data are important to understand the genetic spectrum in defined geographical regions, because publicly available genome information only contains a limited number of genomes and does not reflect all population ancestries. In addition, HANNAH *et al.* [16] reported that PCD is more common than previously assumed, especially in individuals of African ancestry who appear to be under-recognised for PCD.

Next, we investigated whether regional differences in the distribution of PCD genotypes might influence the outcome of non-genetic tests used to diagnose PCD, such as TEM, which is recommended by current guidelines [40, 41]. Overall, 72% of study participants had a genotype associated with hallmark pathognomonic ciliary ultrastructure defects detectable by TEM, consistent with previous reports [3, 42]. Interestingly, we found that this proportion varied from country to country depending on the regional prevalence of distinct PCD gene defects (supplementary figures E2 and E3). For example, in Turkey, where variants in *CCNO* and *DNAH11* (not associated with hallmark ciliary ultrastructure defect) are frequently involved in PCD, TEM failed to diagnose more than half of PCD individuals (53%). Thus, knowledge of the regional distribution of distinct PCD gene variants is important to estimate the sensitivity of a test (*e.g.* TEM) used to diagnose PCD. Previous studies in the USA and UK have reported a higher proportion of hallmark ciliary ultrastructure defects in PCD (from 83% to 86%), which might reflect regional differences in the prevalence of affected PCD genes associated with class I ciliary defects [5, 43]. In some countries, the use of TEM as the first-line diagnostic test, prior to selecting individuals for genetic testing, might result in failure to identify PCD individuals without pathognomonic ciliary ultrastructure

defects. This may also explain the discrepancy in the relative frequency of pathogenic variants detected in similarly sized *DNAH11* and *DNAH5*. The highest prevalence of pathogenic *DNAH11* variants was reported in a study based on variant frequencies available in public databases [16], while in the present cohort, where patients were selected based on regional diagnostic schemes often relying on TEM, the frequency of pathogenic variants in *DNAH11* ($\times 142$) was much lower than *DNAH5* ($\times 275$).

This registry study included participating centres from many countries with different diagnostic resources (e.g. TEM) and expertise. Accordingly, the proportion of PCD individuals with genetic variants associated with hallmark pathognomonic ciliary ultrastructure defects differed considerably among countries (47–100%), likely reflecting distinct regional prevalence of genetic variants. In addition, strategies and techniques used to establish the genetic PCD diagnosis differed among these centres and ranged from targeted Sanger sequencing of individual high-prevalence genes to comprehensive next-generation sequencing. This is a limitation, because it leads to bias in terms of the genes and genetic variants reported. However, our study only reported PCD individuals with a confirmed genetic diagnosis, rendering the diagnostic accuracy very high when compared to other studies where probable PCD diagnoses had been included [44, 45]. Correct interpretation of genetic reports is a common problem [46]. It is even more difficult in rare, genetically and phenotypically heterogeneous diseases such as PCD. The fact that 148 individuals (11% of our cohort) did not meet the ACMG/AMP criteria [14] confirms that genetic PCD diagnosis is very complex, and indicates the need to train specialised respiratory physicians in the interpretation of genetic reports. Genetic diagnosis is increasingly important for patient-centred management in PCD. In other respiratory diseases, such as cystic fibrosis, genetic testing has been instrumental for diagnosis and the development of successful genotype-specific therapies that require recognition of specific pathogenic *CFTR* variants [18, 19, 47–49]. Personalised therapies for PCD, such as gene-specific mRNA replacement, are currently under investigation [50], and the inclusion of PCD individuals in randomised clinical trials will require genetic confirmation of the diagnosis.

The large size of the genotyped PCD cohort in this registry study enabled detailed genotype–phenotype studies. We here investigated the distribution of laterality defects and FEV₁ z-scores in genetically confirmed PCD individuals. The overall proportion of individuals with laterality defects in our cohort was 42%. Laterality defects were only present in individuals with genetic defects known to be associated with laterality defects, confirming the good quality of the genetic diagnosis [1]. Interestingly, several studies reporting situs information in PCD populations showed higher rates of laterality defects [51–53]. Those studies mainly diagnosed PCD by TEM and therefore had a bias to identify more PCD individuals with laterality defects (49–54%), including situs ambiguous (6–12%) [51–53]. Here, we chose genetics for diagnosis and included many PCD types that are not associated with laterality defects. This might explain why we report a higher proportion of PCD individuals with situs solitus (55%) and lower proportions of laterality defects (42%: 39% situs inversus totalis; 3% situs ambiguous). The prevalence of laterality defects differed considerably among countries, reflecting regional distribution of the relevant genotypes. Laterality defects were significantly more frequent in PCD individuals with hallmark pathognomonic ciliary ultrastructure defects than in individuals without hallmark defects, consistent with previous findings in a smaller PCD cohort [3]. It is known that the absence of laterality defects and the lack of pathognomonic ultrastructural ciliary defects make the PCD diagnosis very difficult. [3, 54]. Moreover, PCD individuals without ciliary ultrastructure defects appear to have higher nasal nitric oxide production rates, further hampering PCD diagnosis [3, 6]. Our study confirmed that diagnosis of PCD by standard (non-genetic) tests may be less efficient in populations characterised by a low prevalence of genetic variants causing laterality defects and/or leading to hallmark ciliary ultrastructure defects (such as Turkey in our cohort).

Studies of genotype–phenotype correlations concerning the decline of lung function in PCD have been so far reported only in small genotyped PCD cohorts [4, 5, 10, 55–59]. Analysis of the large cohort in our study demonstrated substantial correlations, indicating that PCD lung function outcomes are related to individual genotypes (figure 5). We showed that the lowest FEV₁ z-scores in the whole PCD cohort were associated with pathogenic variants in *CCNO* (n=25), followed by *CCDC39* (n=64) and *CCDC40* (n=101). Significant genotype–phenotype correlations for *CCNO* and *CCDC40* have not been reported so far. A smaller study has shown a significant reduction of FEV₁ z-score only in individuals with *CCDC39* variants (n=35) [4]. However, the same study only recruited 25 *CCDC40*-variant individuals without significant reduction of FEV₁ scores, possibly due to small sample size. Consistent with our findings, several reports lacking genetic test results have shown a severe reduction of FEV₁ in individuals with microtubular disorganisation and inner dynein arm defects revealed by TEM that are frequently associated with either *CCDC39* or *CCDC40* variants [4, 5, 41, 55, 56, 58, 60]. Interestingly, smaller studies including individuals with microtubular disorganisation and inner dynein arm defects showed heterogeneous results

concerning BMI: a study in 41 PCD individuals in the USA showed a reduction of BMI [56] whereas a study in Italy with 31 individuals showed a normal BMI [36]. In our large cohort comprising 181 PCD individuals, we did not see a significant reduction in BMI.

We observed that the reduction of FEV₁ z-scores was milder in individuals with *DNAH11* variants than in the whole PCD cohort, consistent with findings in a smaller *DNAH11* cohort [4]. FEV₁ z-scores were also significantly higher in individuals with *ODAD1* variants than in the whole cohort, which has not been reported previously. FEV₁ z-scores associated with *DNAH11* or *ODAD1* variants differed among the age groups: their reduction was milder in younger individuals (<60 years old (*DNAH11*) and <30–35 years old (*ODAD1*)), whereas median FEV₁ z-scores were similarly low in older individuals (>60 years old (*DNAH11*) and >30–35 years old (*ODAD1*)) as in the total PCD cohort (figure 5b). Thus, PCD individuals with genotypes associated with a mild reduction of FEV₁ z-scores should be closely monitored. A limitation of this study is the limited or lack of data from older individuals (>50 years old) for most of the gene groups. Larger longitudinal studies investigating the age-dependency of lung function in the different PCD gene groups are needed.

We observed further interesting correlations between genotypes and FEV₁ z-score-associated pulmonary phenotypes, but the respective gene groups were too small for statistical analyses (supplementary table E1). For example, a strong reduction of FEV₁ z-scores was seen in the group of individuals with *MCIDAS* variants (n=5), consistent with a severe ciliogenesis defect, resembling findings in individuals with *CCNO* variants [31–33]. Interestingly, the group of individuals with *RSPH1* variants (n=15) showed a lower median FEV₁ z-score than the total PCD cohort, suggesting that the respiratory disease course in these individuals might not be as subtle as previously reported [61]. This is also consistent with the FEV₁ z-scores in individuals with pathogenic variants in genes encoding other radial spoke head proteins (*RSPH4A*, *RSPH9*, *RSPH3*; supplementary table E1).

In conclusion, we demonstrated that a high proportion of PCD individuals are difficult to diagnose due to the absence of pathognomonic defects of the ciliary ultrastructure and absence of laterality defects, confirming the importance of genetic testing for PCD diagnosis. The unprecedented use of a multinational dataset of DNA variants and clinical characteristics in PCD individuals allowed us to reveal substantial correlations of genotypes with FEV₁ z-scores, suggesting that genetic diagnosis might help to predict the clinical prognosis in affected individuals. Individuals with pathogenic variants in certain genes (*CCNO*, *CCDC39* and *CCDC40*) may require more rigorous and intensive clinical management.

Acknowledgements: The authors thank the individuals with PCD and their families for participating in the studies and especially acknowledge the national patient support groups. Several participating centres are healthcare providers in the European Reference Network ERN-LUNG and/or are members of BEAT-PCD clinical research collaboration supported by the European Respiratory Society. For excellent organisational assistance, the authors thank the study nurses S. Helms and M. Tekaar (Department of General Pediatrics, University Hospital Muenster, Muenster, Germany). For their excellent technical assistance, the authors thank M. Herting and L. Schwidessen (Department of General Pediatrics, University Hospital Muenster, Muenster, Germany), C. Westermann (Gerhard Domagk Institute of Pathology, University Hospital Muenster, Muenster, Germany) and K. Wohlgemuth, A. Borgscheiper and S. Sivalingam (Department of General Pediatrics, University Hospital Muenster, Muenster, Germany). We thank G.W. Dougherty for editing the manuscript and C. Rieck for assistance with the design of the graphical abstract (both Department of General Pediatrics, University Hospital Muenster, Muenster, Germany). The authors thank the following persons for substantial support in this study: N.T. Loges, S. Freischem, L. Biebach, J. Wallmeier, A. Schramm and R.D. Tenardi (Department of General Pediatrics, University Hospital Muenster, Muenster, Germany), A. Moreno-Galdó (Paediatric Pulmonology Section, Department of Paediatrics, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Universitat Autònoma de Barcelona, Spain; and Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain), J. Korfmacher (Department of Pediatrics, Faculty of Medicine and University Hospital, University of Cologne, Cologne, Germany), S. Scigliano (Respiratory Center, Ricardo Gutiérrez Children's Hospital, Buenos Aires, Argentina), F. Santamaria (Department of Translational Medical Sciences, Pediatric Pulmonology, Federico II University, Naples, Italy), R. Cutrera and A. Allegorico (Pneumology and Cystic Fibrosis Unit, Academic Department of Pediatrics, Bambino Gesù Children's Hospital, Rome, Italy), P. Pohunek and L. Borek-Dohalska (Department of Paediatrics, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic), A. Holubova (Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic), B. Karadag (Department of Pediatric Pulmonology, Marmara University School of Medicine, Istanbul, Turkey), M. Dotan (Pulmonary Institute, Schneider Children's Medical Center of Israel, Petach-Tikva, Israel; and Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel), D. Prais

(Pulmonary Institute, Schneider Children's Medical Center of Israel, Petach-Tikva, Israel; and Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel), E. Rietschel (Department of Pediatrics, Faculty of Medicine and University Hospital, University of Cologne, Cologne), F. Brinkmann (University Children's Hospital, Ruhr University Bochum, Katholisches Klinikum Bochum, Bochum, Germany) and D.J. Morris-Rosendahl (Clinical Genetics and Genomics, Royal Brompton Hospital, Guy's and St Thomas' NHS Foundation Trust and NHLI, Imperial College London, London, UK). The graphical abstract was created with BioRender.com. The authors would like to thank J. Strub and the varSEAK Team as well as the groups that provided their variant data.

Ethics statement: The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the Westphalian Wilhelms-University of Muenster (Muenster, Germany; AZ 2011-270-f-S). Ethical approval was obtained from the institutional ethics and/or national research committees of each centre involved in the study. Each participant or his or her legal guardian gave written informed consent prior to participation.

The study is registered on ClinicalTrials.gov (NCT:04717115).

Conflict of interest: The authors have no potential conflicts of interest to disclose.

Support statement: This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG OM6/7, OM6/8, OM6/10, OM6/14, CRU 326 (to H. Omran, J. Raidt); OL 450/1 (H. Olbrich); CRC 1449 (project 431232613; sub-project Z02 to M.A. Mall)), the Interdisziplinäres Zentrum für Klinische Forschung Muenster (Om2/009/12, Om2/015/16 and Om2/010/20), Registry Warehouse (Horizon2020 GA 777295) and the German Federal Ministry of Education and Research (82DZL009B1 to M.A. Mall). J.F. Roehmel is a participant of the Case Analysis and Decision Support (CADS) programme funded by the Berlin Institute of Health. The authors thank the "Børnelungefonden" and "Rigshospitalet's research fund" (K.G. Nielsen). A. Shoemark is supported by Asthma and Lung UK. The National UK PCD service is supported by NHS England. Some study authors and data contributors participate in the BEAT-PCD clinical research collaboration supported by the European Respiratory Society. This study was supported and promoted as part of work package 3 during the 2020-2023 BEAT-PCD funding period. H.M. Mitchison is supported by the NIHR Biomedical Research Centre at Great Ormond Street Hospital. S. Rovira-Amigo was funded by a grant from Instituto de Salud Carlos III (ISCIII) through the project "PI20/01419" and co-funded by the EU and a grant of the Spanish Society of Paediatrics (Invest-AEP 2021). V. Martinu was supported by Ministry of Health of the Czech Republic, grant number NV19-07-00210 and supported by Motol University Hospital, Prague, Czech Republic, 00064203 (conceptual development of research). E. Ziętkiewicz was supported by the Polish National Science Centre, grant 2018/31/B/NZ2/03248. Funding information for this article has been deposited with the Crossref Funder Registry.

References

- Wallmeier J, Nielsen KG, Kuehni CE, *et al.* Motile ciliopathies. *Nat Rev Dis Primers* 2020; 6: 77.
- Pennekamp P, Raidt J, Wohlgemuth K, *et al.* Primary ciliary dyskinesia. In: Wagner TOF, Humbert M, Wijsenbeek M, *et al.*, eds. Rare Diseases of the Respiratory System. Sheffield, European Respiratory Society, 2023; pp. 118–134.
- Raidt J, Krenz H, Tebbe J, *et al.* Limitations of nasal nitric oxide measurement for diagnosis of primary ciliary dyskinesia with normal ultrastructure. *Ann Am Thorac Soc* 2022; 19: 1275–1284.
- Shoemark A, Rubbo B, Legendre M, *et al.* Topological data analysis reveals genotype–phenotype relationships in primary ciliary dyskinesia. *Eur Respir J* 2021; 58: 2002359.
- Kinghorn B, Rosenfeld M, Sullivan E, *et al.* Airway disease in children with primary ciliary dyskinesia: impact of ciliary ultrastructure defect and genotype. *Ann Am Thorac Soc* 2023; 20: 539–547.
- Legendre M, Thouvenin G, Taytard J, *et al.* High nasal nitric oxide, cilia analyses, and genotypes in a retrospective cohort of children with primary ciliary dyskinesia. *Ann Am Thorac Soc* 2022; 19: 1704–1712.
- Emiralioglu N, Taşkıran EZ, Koşukcu C, *et al.* Genotype and phenotype evaluation of patients with primary ciliary dyskinesia: first results from Turkey. *Pediatr Pulmonol* 2020; 55: 383–393.
- Fassad MR, Patel MP, Shoemark A, *et al.* Clinical utility of NGS diagnosis and disease stratification in a multiethnic primary ciliary dyskinesia cohort. *J Med Genet* 2020; 57: 322–330.
- Yiallourous PK, Kouis P, Kyriacou K, *et al.* Implementation of multigene panel NGS diagnosis in the national primary ciliary dyskinesia cohort of Cyprus: an island with a high disease prevalence. *Hum Mutat* 2021; 42: e62–e77.
- Rumman N, Fassad MR, Driessens C, *et al.* The Palestinian primary ciliary dyskinesia population: first results of the diagnostic and genetic spectrum. *ERJ Open Res* 2023; 9: 00714–2022.
- Werner C, Lablans M, Ataian M, *et al.* An international registry for primary ciliary dyskinesia. *Eur Respir J* 2016; 47: 849–859.

- 12 Ardura-Garcia C, Goutaki M, Carr SB, *et al.* Registries and collaborative studies for primary ciliary dyskinesia in Europe. *ERJ Open Res* 2020; 6: 00005-2020.
- 13 Raidt J, Maitre B, Pennekamp P, *et al.* The disease-specific clinical trial network for primary ciliary dyskinesia: PCD-CTN. *ERJ Open Res* 2022; 8: 00139-2022.
- 14 Richards S, Aziz N, Bale S, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405-424.
- 15 Shoemark A, Boon M, Brochhausen C, *et al.* International consensus guideline for reporting transmission electron microscopy results in the diagnosis of primary ciliary dyskinesia (BEAT PCD TEM Criteria). *Eur Respir J* 2020; 55: 1900725.
- 16 Hannah WB, Seifert BA, Truty R, *et al.* The global prevalence and ethnic heterogeneity of primary ciliary dyskinesia gene variants: a genetic database analysis. *Lancet Respir Med* 2022; 10: 459-468.
- 17 Horneff N, Olbrich H, Horvath J, *et al.* *DNAH5* mutations are a common cause of primary ciliary dyskinesia with outer dynein arm defects. *Am J Respir Crit Care Med* 2006; 174: 120-126.
- 18 Ong T, Ramsey BW. Cystic fibrosis: a review. *JAMA* 2023; 329: 1859-1871.
- 19 Bell SC, Mall MA, Gutierrez H, *et al.* The future of cystic fibrosis care: a global perspective. *Lancet Respir Med* 2020; 8: 65-124.
- 20 Zariwala MA, Leigh MW, Ceppa F, *et al.* Mutations of *DNAI1* in primary ciliary dyskinesia: evidence of founder effect in a common mutation. *Am J Respir Crit Care Med* 2006; 174: 858-866.
- 21 Antony D, Becker-Heck A, Zariwala MA, *et al.* Mutations in *CCDC39* and *CCDC40* are the major cause of primary ciliary dyskinesia with axonemal disorganization and absent inner dynein arms. *Hum Mutat* 2013; 34: 462-472.
- 22 Onoufriadis A, Paff T, Antony D, *et al.* Splice-site mutations in the axonemal outer dynein arm docking complex gene *CCDC114* cause primary ciliary dyskinesia. *Am J Hum Genet* 2013; 92: 88-98.
- 23 Kos R, Israëls J, van Gogh CDL, *et al.* Primary ciliary dyskinesia in Volendam: diagnostic and phenotypic features in patients with a *CCDC114* mutation. *Am J Med Genet C Semin Med Genet* 2022; 190: 89-101.
- 24 Djakow J, Kramná L, Dušátková L, *et al.* An effective combination of Sanger and next-generation sequencing in diagnostics of primary ciliary dyskinesia. *Pediatr Pulmonol* 2016; 51: 498-509.
- 25 Knowles MR, Leigh MW, Ostrowski LE, *et al.* Exome sequencing identifies mutations in *CCDC114* as a cause of primary ciliary dyskinesia. *Am J Hum Genet* 2013; 92: 99-106.
- 26 Panizzi JR, Becker-Heck A, Castleman VH, *et al.* *CCDC103* mutations cause primary ciliary dyskinesia by disrupting assembly of ciliary dynein arms. *Nat Genet* 2012; 44: 714-719.
- 27 Shoemark A, Moya E, Hirst RA, *et al.* High prevalence of *CCDC103* p.His154Pro mutation causing primary ciliary dyskinesia disrupts protein oligomerisation and is associated with normal diagnostic investigations. *Thorax* 2018; 73: 157-166.
- 28 Olbrich H, Schmidts M, Werner C, *et al.* Recessive *HYDIN* mutations cause primary ciliary dyskinesia without randomization of left-right body asymmetry. *Am J Hum Genet* 2012; 91: 672-684.
- 29 Zietkiewicz E, Bukowy-Bieryllo Z, Rabiasz A, *et al.* *CFAP300*: mutations in Slavic patients with primary ciliary dyskinesia and a role in ciliary dynein arms trafficking. *Am J Respir Cell Mol Biol* 2019; 61: 440-449.
- 30 Höben IM, Hjej R, Olbrich H, *et al.* Mutations in *C11orf70* cause primary ciliary dyskinesia with randomization of left/right body asymmetry due to defects of outer and inner dynein arms. *Am J Hum Genet* 2018; 102: 973-984.
- 31 Wallmeier J, Al-Mutairi DA, Chen CT, *et al.* Mutations in *CCNO* result in congenital mucociliary clearance disorder with reduced generation of multiple motile cilia. *Nat Genet* 2014; 46: 646-651.
- 32 Amirav I, Wallmeier J, Loges NT, *et al.* Systematic analysis of *CCNO* variants in a defined population: implications for clinical phenotype and differential diagnosis. *Hum Mutat* 2016; 37: 396-405.
- 33 Boon M, Wallmeier J, Ma L, *et al.* *MCIDAS* mutations result in a mucociliary clearance disorder with reduced generation of multiple motile cilia. *Nat Commun* 2014; 5: 4418.
- 34 Mazor M, Alkrinawi S, Chalifa-Caspi V, *et al.* Primary ciliary dyskinesia caused by homozygous mutation in *DNAL1*, encoding dynein light chain 1. *Am J Hum Genet* 2011; 88: 599-607.
- 35 Yiallourous PK, Kouis P, Pirpa P, *et al.* Wide phenotypic variability in *RSPH9*-associated primary ciliary dyskinesia: review of a case-series from Cyprus. *J Thorac Dis* 2019; 11: 2067-2075.
- 36 Pifferi M, Bush A, Mulé G, *et al.* Longitudinal lung volume changes by ultrastructure and genotype in primary ciliary dyskinesia. *Ann Am Thorac Soc* 2021; 18: 963-970.
- 37 Frommer A, Hjej R, Loges NT, *et al.* Immunofluorescence analysis and diagnosis of primary ciliary dyskinesia with radial spoke defects. *Am J Respir Cell Mol Biol* 2015; 53: 563-573.
- 38 Boaretto F, Snijders D, Salvoro C, *et al.* Diagnosis of primary ciliary dyskinesia by a targeted next-generation sequencing panel: molecular and clinical findings in Italian patients. *J Mol Diagn* 2016; 18: 912-922.
- 39 Moore DJ, Onoufriadis A, Shoemark A, *et al.* Mutations in *ZMYND10*, a gene essential for proper axonemal assembly of inner and outer dynein arms in humans and flies, cause primary ciliary dyskinesia. *Am J Hum Genet* 2013; 93: 346-356.

- 40 Lucas JS, Barbato A, Collins SA, *et al.* European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. *Eur Respir J* 2017; 49: 1601090.
- 41 Shapiro AJ, Davis SD, Polineni D, *et al.* Diagnosis of primary ciliary dyskinesia. an official American Thoracic Society clinical practice guideline. *Am J Respir Crit Care Med* 2018; 197: e24–e39.
- 42 Kouis P, Yiallourou PK, Middleton N, *et al.* Prevalence of primary ciliary dyskinesia in consecutive referrals of suspect cases and the transmission electron microscopy detection rate: a systematic review and meta-analysis. *Pediatr Res* 2017; 81: 398–405.
- 43 Shah A, Shoemark A, MacNeill SJ, *et al.* A longitudinal study characterising a large adult primary ciliary dyskinesia population. *Eur Respir J* 2016; 48: 441–450.
- 44 Goutaki M, Pedersen ESL. Phenotype–genotype associations in primary ciliary dyskinesia: where do we stand? *Eur Respir J* 2021; 58: 2100392.
- 45 Halbeisen FS, Jose A, de Jong C, *et al.* Spirometric indices in primary ciliary dyskinesia: systematic review and meta-analysis. *ERJ Open Res* 2019; 5: 00231–2018.
- 46 Sayitoğlu M. Clinical interpretation of genomic variations. *Turk J Haematol* 2016; 33: 172–179.
- 47 Mall MA, Mayer-Hamblett N, Rowe SM. Cystic fibrosis: emergence of highly effective targeted therapeutics and potential clinical implications. *Am J Respir Crit Care Med* 2020; 201: 1193–1208.
- 48 Barry PJ, Mall MA, Álvarez A, *et al.* Triple therapy for cystic fibrosis *Phe508del*-gating and -residual function genotypes. *N Engl J Med* 2021; 385: 815–825.
- 49 Middleton PG, Mall MA, Dřevínek P, *et al.* Elexacaftor-tezacaftor-ivacaftor for cystic fibrosis with a single *Phe508del* allele. *N Engl J Med* 2019; 381: 1809–1819.
- 50 Paff T, Omeran H, Nielsen KG, *et al.* Current and future treatments in primary ciliary dyskinesia. *Int J Mol Sci* 2021; 22: 9834.
- 51 Kennedy MP, Omeran H, Leigh MW, *et al.* Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. *Circulation* 2007; 115: 2814–2821.
- 52 Shapiro AJ, Davis SD, Ferkol T, *et al.* Laterality defects other than situs inversus totalis in primary ciliary dyskinesia: insights into situs ambiguus and heterotaxy. *Chest* 2014; 146: 1176–1186.
- 53 Barber AT, Shapiro AJ, Davis SD, *et al.* Laterality defects in primary ciliary dyskinesia: relationship to ultrastructural defect or genotype. *Ann Am Thorac Soc* 2023; 20: 397–405.
- 54 Kuehni CE, Frischer T, Strippoli MP, *et al.* Factors influencing age at diagnosis of primary ciliary dyskinesia in European children. *Eur Respir J* 2010; 36: 1248–1258.
- 55 Pifferi M, Bush A, Mariani F, *et al.* Lung function longitudinal study by phenotype and genotype in primary ciliary dyskinesia. *Chest* 2020; 158: 117–120.
- 56 Davis SD, Rosenfeld M, Lee HS, *et al.* Primary ciliary dyskinesia: longitudinal study of lung disease by ultrastructure defect and genotype. *Am J Respir Crit Care Med* 2019; 199: 190–198.
- 57 Frija-Masson J, Bassinet L, Honoré I, *et al.* Clinical characteristics, functional respiratory decline and follow-up in adult patients with primary ciliary dyskinesia. *Thorax* 2017; 72: 154–160.
- 58 Davis SD, Ferkol TW, Rosenfeld M, *et al.* Clinical features of childhood primary ciliary dyskinesia by genotype and ultrastructural phenotype. *Am J Respir Crit Care Med* 2015; 191: 316–324.
- 59 Roehmel JF, Doerfler FJ, Koerner-Rettberg C, *et al.* Comparison of the lung clearance index in preschool children with primary ciliary dyskinesia and cystic fibrosis. *Chest* 2022; 162: 534–542.
- 60 Irving S, Dixon M, Fassad MR, *et al.* Primary ciliary dyskinesia due to microtubular defects is associated with worse lung clearance index. *Lung* 2018; 196: 231–238.
- 61 Knowles MR, Ostrowski LE, Leigh MW, *et al.* Mutations in *RSPH1* cause primary ciliary dyskinesia with a unique clinical and ciliary phenotype. *Am J Respir Crit Care Med* 2014; 189: 707–717.