Case Report

GENETICS

Primary ciliary dyskinesia: a case report of double DNAH11 mutant alleles

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Abstract

Primary ciliary dyskinesia (PCD), a rare disorder, is genetically varied. Mutations in proteins involved in the structure, function, or assembly of cilia are known to determine situs inversus, male infertility, and chronic destructive airway disease. PCD is inherited by an autosomal recessive pattern of inheritance in most cases. Nonetheless, patterns of autosomal dominant and X-linked inheritance have been mentioned. A history of recurrent upper and lower respiratory tract infections raised clinical suspicion of primary ciliary dyskinesia in a 10-year-old patient. Genetic tests were performed using next-generation sequencing technology (Illumina NextGen) with the multiplex ligation-dependent probe amplification technique for primary ciliopathies and syndromes subject to differential diagnosis. Genetic testing identified two pathogenic variants, not previously associated with a case report in the literature, c.7727A>G (p.Asp2576Gly) and c.8578G>A (p.Gly2860Ser), within the *DNAH11 gene*, which is associated with autosomal recessive PCD. The result also reported mutations in other genes involved in autosomal recessive PCD (*DNAH8*, *DNAH9* and *ZMYND10*), which were classified as variants with uncertain clinical significance. Transmission electron microscopy of respiratory cilia and nasal nitric oxide measurement cannot be used to diagnose PCD in patients with *DNAH11* mutations because the structure of cilia is normal, and the levels of NO are not constantly low. High-speed video microscopy analysis can be helpful because *DNAH11* mutations cause a distinct phenotype of PCD. Nevertheless, the mutation analysis of various PCD-causing genes remains the easiest to conduct and with good results. Genetic research on PCD has identified a number of significant ciliary genes in recent years, offering fresh perspectives on the molecular processes underlying cilia assembly and function. This facilitates the development of new methods for the diagnosis, prevention, and treatment of PCD. However, because it is a highly complex and heterogeneous disease, the field of gene diagnosis and therapy in PCD is still in its infancy.

Keywords: primary ciliary dyskinesia, *DNAH11*, *DNAH8*, *DNAH9*, *ZMYND10*

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Introduction

Primary ciliary dyskinesia (PCD) is a rare, genetically heterogeneous disorder [1]. Dextrocardia, with or without situs inversus totalis, is present in 50% of individuals with PCD; in this instance, the condition is also known as Kartagener syndrome. The prevalence of PCD was thought to be 1 in 20,000 – 60,000 [2,3], but recent studies have demonstrated a higher prevalence of the disease in the population of 1 in 7,500 [4]. Advances in sequencing technology, such as whole exome sequencing, massively parallel sequencing and functional and proteomic studies have allowed international collaboratives to identify new genes associated with PCD [1,2].

At present, more than 56 genes have been linked to PCD, and this number is likely to grow with future discoveries [4,5]. Numerous genes oversee certain

ultrastructural elements, such as the proteins found in the central apparatus, radial spokes, inner and outer dynein arms, and dynein regulatory complex [4,5]. More recently, mutations in genes coding for cytoplasmic proteins, which are not an integral part of the cilia axoneme but are essential for the preassembly of dynein motor units, have also been found to cause disease [5-7].

Autosomal recessive inheritance patterns are typical in cases of PCD, although X-linked and autosomal dominant patterns are also known [5,6]. The most prevalent types of mutations in western nations are those in the *DNAH5*, *DNAH11*, *DNAI1*, *CCDC39*, *CCDC40*, and *HYDIN* [7,8]. *DNAH11* was first identified in 2002 in a patient whose ciliary ultrastructure appeared normal under transmission electron microscopy (TEM) [9,10]. Mutations of certain genes, such as *DNAH11* that encodes the dynein protein of the outer arm, do not seem to affect the ultrastructure of the cilia evident by TEM, remaining normal, but studies using high-speed video microscopy (HSVM) show that their function is altered causing rapid movements with small amplitude [5,10]. Clinical studies have revealed that *DNAH11* mutations can result in a range of organ laterality defects, such as situs ambiguous, in both human and murine models [4,11].

Case Report

The case reported in the present study is of a 10-year-old female with various ENT (Ear Nose and Throat) related conditions. The patient is the single child of a non-consanguineous couple with physiological postnatal overall development. Starting at the age of 3, the girl presented recurrent rhinosinusal and otic inflammatory manifestations. At the age of 5, chronic serous otitis complications required transtympanic aerator implants and a nasal polypectomy procedure to ameliorate the symptoms. Furthermore, the patient had multiple episodes of bronchitis and bronchiolitis, for which she was further investigated in the Pneumology Clinic over the years. Several biological markers, including a normal sweat test, and allergy-related indicators such as skin prick tests and plasma interleukins, were assessed. While there were some nonspecific changes noted in allergy tests for pollen and mites, no abnormalities indicating airway diseases were detected. Over the years, the patient was treated with antibiotics, mucolytics, broncho-dilators, oronasal and systemic antiallergic drugs.

Because of this medical history (Table I), the clinical suspicion of PCD was raised. On the recommendation of the geneticist, the patient underwent specific testing for primary ciliopathies and syndromes that are the subject of differential diagnosis. The molecular analysis was based on Next Generation Sequencing technology (Illumina NextGen) in combination with MLPA (Multiplex Ligation-Dependent Probe Amplification) technique, which detects both structural abnormalities and deletions or duplications at the level of the most frequent 43 genes (*AK7*, *ARMC4*, *C11orf70*, *CCDC103*, *CCDC114*, *CCDC151*, *CCDC39*, *CCDC40*, *CCDC65*, *CCNO*, *CEP164*, *CFAP298*, *CFTR*, *DNAAF1*, *DNAAF2*, *DNAAF3*, *DNAAF4*, *DNAAF5*, *DNAH1*, *DNAH11*, *DNAH5*, *DNAH8*, *DNAH9*, *DNAI1*, *DNAI2*, *DNAJB13*, *DNAL1*, *DRC1*, *GAS8*, *LRRC56*, *LRRC6*, *MCIDAS*, *NOTCH2*, *OFD1*, *PIH1D3*, *RPGR*, *RSPH1*, *RSPH3*, *RSPH4A*, *RSPH9*, *SERPINA1*, *SPAG1*, *ZMYND10*) associated with the occurrence of primary ciliopathies and differential diagnoses, including cystic fibrosis. Genomic DNA was extracted from peripheral venous blood and amplified using primers specific to regions of interest and positive and negative controls for each individual reaction.

Table I. Clinical features of the present case in comparison with other reported cases of patients with PCD.

Phenotype in PCD	Literature reports on PCD phenotype	Patient Phenotype
Pulmonary disorders	Neonatal respiratory distress [12-14]	n/a[1]
	Airway infection [12-14]	re[2] of Bronchiolitis and Bronchitis
	Chronic cough [12-14]	Present
	Pneumonia [13]	One episode
	Atypical Asthma [12-14]	Absent
Ear disorders	Chronic otitis media [12-14]	Chronic serous otitis media
	Hearing loss [12,13]	Absent
Nose and paranasal disorders	Chronic rhinosinusitis [13]	re[2] of Acute rhinosinusitis
	Chronic sinusitis [12,13]	Absent
	Nasal polyposis [13]	Nasal polyps removed (age of 3)
Genitourinary disorders	Infertility [12,13]	n/a[1]
Situs disorders	Situs inversus totalis [12-14]	Absent
	Heterotaxy [12,13]	Absent
Eye disorders	Retinitis pigmentosa [13]	Absent
Gastrointestinal disorders	Esophageal atresia [13,14]	Absent
	Biliary atresia [13,14]	Absent
Nervous disorders	Hydrocephalus [14]	Absent
Cardiovascular disorders	Complex congenital heart defects [13,14]	Absent

[1] n/a – not available data; [2] re – recurrent episodes

The molecular analysis revealed two heterozygous pathogenic variants, *c.7727A>G (p.Asp2576Gly)* and *c.8578G>A (p.Gly2860Ser)*, in the *DNAH11* gene. The first mutation in the 47th exon replaces aspartic acid, acidic and polar, with glycine, neutral and non-polar, at codon 2576 of the DNAH11 protein (p.Asp2576Gly). The second mutation in the 52nd exon replaces glycine, neutral and non-polar, with serine, neutral and polar, at codon 2860 of the DNAH11 protein (p.Gly2860Ser).

No case report has yet been published in the literature that describes these mutations in *DNAH11* gene. Advanced modelling of protein sequence and biophysical properties, such as structural, functional, and spatial information, amino acid conservation, physicochemical variation, residue mobility, and thermodynamic stability, performed at by in silico models, indicates that these missense variants are expected to disrupt DNAH11 protein function. Family studies suggest that these variants are likely on opposite chromosomes, both parents undergoing the same sequencing tests confirming that *c.7727A>G (p.Asp2576Gly)* mutation is present in the mother's genome and the *c.8578G>A (p.Gly2860Ser)* mutation being a part of the father's genome.

In addition, three de novo heterozygous mutations with uncertain significance were found in *DNAH8*, *DNAH9*, and *ZMYND10* (which have been associated with PCD pathogenesis in previous literature), following the completion of the genetic testing in the current patient's case. The *DNAH8* identified mutation resulted in the substitution of arginine with glycine at codon 3247 of the DNAH8 protein (p.Arg3247Gly). The *DNAH9* identified mutation substituted methionine with valine at codon 4113 of the DNAH9 protein (p.Met4113Val). The final mutation detected in this patient's case was in the gene *ZMYND10* and resulted in the replacement of leucine with valine at codon 188 of the ZMYND10 protein (p.Leu188Val).

Currently, the patient is supervised by a team consisting of pulmonologists and ENT specialists.

Discussion

Comprising various factors, respiratory ciliopathies are intricate pulmonary disorders exhibiting genetic variation while sharing a similar respiratory manifestation. With a well-established and acknowledged diagnostic route, PCD continues to be the most understood ciliopathy [15]. The course of the disease varies greatly; whereas some individuals have worse outcomes, others maintain a comparatively decent quality of life and lung function well into later adulthood [15,16]. There is evidence that distinct gene mutations result in diverse phenotypes, variations in some genes being more likely to induce infertility, whereas other genes are never linked to situs abnormalities [17]. There are still scarce data, despite a few studies suggesting a link between specific genes and the severity of pulmonary disease [16]. Moreover, most patients are reportedly seen by a physician more than 50 times before a diagnosis is made, at a mean age of 10.9 years [18]. For certain patients, identifying a gene may aid in the identification of a specific phenotype and be useful in clinical evaluation and therapeutic planning [15].

The clinical features of PCD are nowadays well recognized, but clinical suspicion of the disease in the absence of heterotaxy remains rather low, although first manifestations may be present from infancy. The primary care clinician should know the clinical spectrum of this condition to select appropriately the children who need further investigation for the diagnosis of PCD [19].

The first mutation reported in the *DNAH11* had been identified in a patient who had both PCD symptoms and a genetic diagnosis of cystic fibrosis with normal ciliary ultrastructure [20]. Subsequent reports conclusively demonstrated that mutant *DNAH11* causes PCD in patients with normal ultrastructure [19].

Currently, there is no established gold standard for diagnosing PCD according to the guidelines outlined by European Respiratory Society (ERS) [21]. However, utilizing TEM to detect structural abnormalities in respiratory cilia or/and confirmed genetic abnormalities represents one of the most used diagnostic approaches. Also, it has been previously believed that normal ciliary ultrastructure or mild non-diagnostic alterations are observed in 22–72% of PCD patients [11]. Nonetheless, recent literature indicates that 82% of PCD patients exhibit normal ciliary ultrastructure [22]. This means that it is necessary to do immunofluorescent (IF) labelling of axonemal proteins, digital high-speed videomicroscopy (DHSV), or nasal nitric oxide (NO) testing to clearly establish ciliary failure. Other investigators have also reported this, especially with the hyperkinetic phenotype of *DNAH11* mutations observed with the help of DHSV. Nasal NO values are generally low but can fall within the normal range, similar to non-PCD patients [23,24]. IF microscopy with anti-DNAH11 antibodies can identify some individuals with primary ciliary dyskinesia who have mutations in DNAH11, yet its effectiveness is limited due to the increasing number of newly identified mutations and those that are currently unknown, as demonstrated in the present case [25]. Although these techniques might be useful for PCD diagnosis, they have limitations and need to be supported by new methodologies, including genetic analysis of genes related to PCD [11,25]. Moreover, in Romania, TEM is typically used only for research purposes and not for diagnostics, as well as DHSV, NO measurement and IF microscopy. Genome sequencing remains the only solution for diagnosing this disease, but multiple limitations exist due to the complexity of cilia, which involve more than 200 different polypeptides. The diversity of mutations, coupled with the phenomenon that multiple mutations can lead to the same altered phenotype, makes this conception even more complex [24,25].

This case introduces into the literature two inherited *DNAH11* mutations, namely c.7727A>G (p.Asp2576Gly) and c.8578G>A (p.Gly2860Ser), which have not been documented in any prior case reports in the literature. These two mutant alleles are accountable for the symptoms described earlier and for the PCD diagnosis assigned to this patient.

Besides those, three de novo mutations in the *DNAH8*, *DNAH9* and *ZMYND10* genes were identified. The mutant DNAH8 protein (p.Arg3247Gly) has a frequency of 0.1% and the DNAH9 protein (p.Met4113Val) has a frequency of 0.002% in population databases (gnomAD). Although these mutations have been observed in the general population, they have not been linked to *DNAH8*/*DNAH9* PCD case reports in literature. The present mutant ZMYND10 protein (p.Leu188Val) is not present in population databases (gnomAD no frequency). Predictive algorithms, such as SIFT, PolyPhen-2, and Align-GVGD, all indicate that those variants are likely to be tolerated. Overall, the current data is insufficient to determine the role of these three de novo mutations in the pathogenesis of PCD. Therefore, they have been classified as variants of uncertain significance. It is worth noting that a notable fraction of the outbred population presents a single mutant gene associated with a PCD variant without manifesting PCD symptoms [4]. Consequently, the mutations identified in the *ZMYND10*, *DNAH8*, and *DNAH9* genes should not be attributed as causative factors of PCD in this instance. Their inclusion serves to provide a more comprehensive characterization of the presented case and to underscore the concept that within the population, individuals may harbour mutations in specific key genes linked to PCD without experiencing illness.

Genetic counseling is crucial for offering comprehensive support to families and individuals as they manage this complex genetic condition [8]. Through genetic counseling, patients gain a clearer understanding of inheritance patterns, potential genetic variations, and associated health risks. In addition, geneticists offer priceless information on available reproductive choices, treatment alternatives, and diagnostic testing, enabling people to make well-informed decisions regarding their health and family planning [8,16]. Genetic counselling facilitates proactive treatment strategies and optimal health outcomes for persons with PCD and their families by combining medical expertise with personalized assistance [16].

In **conclusion**, PCD is a genetically heterogeneous disorder that affects cilia function and structure. This research presents a case study of a 10-year-old female patient with a history of multiple pulmonary diseases and chronic or recurrent ear infections which determined a general mild phenotype. The patient was diagnosed using Next-generation sequencing (NGS) panel test of the known genes which are implicated in the pathogenesis of PCD. The patient inherited two pathogenic variants, not previously associated with a case report in the literature, c.7727A>G (p.Asp2576Gly) and c.8578G>A (p.Gly2860Ser), in the *DNAH11* gene from her parents (both being heterozygous). Additionally, there were other de novo mutations found in the *DNAH8*, *DNAH9*, and *ZMYND10* genes, but their significance as variants remains uncertain.

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