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A Deep Intronic, Pathogenic Variant in *DNAH11* Causes Primary Ciliary Dyskinesia

To the Editor:

Primary ciliary dyskinesia (PCD, OMIM#244400) is a rare respiratory disease resulting in chronic oto-sino-pulmonary disease, bronchiectasis, infertility, and organ laterality defects. Classically investigated through ciliary ultrastructural analysis on transmission electron microscopy (TEM), immunofluorescent (IF) staining of ciliary proteins, and genetic testing, PCD is difficult to diagnose as these investigations may be inconclusive, though low nasal nitric oxide (nNO) may bolster diagnostic suspicion of PCD (1, 2). Next generation sequencing and whole exome sequencing technologies have expanded the genetic basis for PCD to >50 different genes, mostly with autosomal recessive trait (3, 4). Current genetic testing fails to diagnose 20–30% of probable PCD cases (5), but the diagnostic contribution from genetic analysis of noncoding (intronic) regions has not been extensively evaluated in PCD.

DNAH11 (dynein axonemal heavy chain 11, OMIM#603339) is a common PCD-related gene, encoding an outer dynein arm protein. Yet, TEM in *DNAH11*-associated cases is normal with intact dynein arms (6). *DNAH11* is difficult to detect on IF testing, often leaving low nNO as the sole diagnostic clue (3, 7). In one cohort, 25% of unsolved PCD cases with normal TEM harbored monoallelic, pathogenic variants in *DNAH11*, without a second disease-causing *DNAH11* variant (6). The recent report of a deep intronic, disease-causing variant in *DNAH11* at c.6547–963G > A (8) led us to test for this variant in our probable PCD population.

Recruited under the auspices of Genetic Disorders of Mucociliary Clearance Consortium (GDMCC), we investigated 22 unrelated cases with clinical PCD (compatible phenotype and low nNO when available), but non-informative TEM and non-diagnostic genetic testing, with only 1 pathogenic/likely pathogenic variant identified in *DNAH11* (NM_001277115.1). These cases underwent targeted screening for the recently identified *DNAH11* deep intronic variant c.6547–963G > A (8), while further whole genome sequencing was not performed.

Human study protocols were approved by site Institutional Review Boards. Polymerase chain reaction on genomic DNA was performed at 60°C annealing temperature using a described protocol (9). The M13-tagged gene-specific primers 5′-M13-AGAGGATGGCAGTATATGGAAC-3′ (forward) and 5′-M13-TAGAGACCAGGGAGGTTGCT-3′ (reverse) were used for amplification followed by direct Sanger sequencing using M13 primers or by restriction-enzyme (*BPU10I* from New England Biolabs) digestion per the manufacturer's instructions, following product visualization by 2% agarose gel electrophoresis. The resulting amplicon (729-bp) upon restriction digestion is expected to abrogate the *BPU10I* site in the presence of a c.6547–963G > A heterozygous variant yielding 4 fragments (621-bp, 360-bp, 261-bp, and 108-bp) versus 3 fragments (360-bp, 261-bp, and 108-bp) for the wild-type.

The c.6547–963G > A deep intronic variant was identified in one French-Canadian adolescent female also harboring a canonical splice-donor site variant c.5778 + 1G > A (p.splice), previously found on clinical multigene testing (plus one pathogenic *CFTR* variant, with negative sweat chloride testing). Parental segregation revealed the proband's father and mother were carriers of c.5778 + 1G > A (p.splice) and c.6547–963G > A variants, respectively (Figure 1). This patient had classic PCD symptoms, including neonatal respiratory distress, year-round wet cough and nasal congestion from birth, and recurrent bronchitis, sinusitis, and otitis but lacked bronchiectasis or laterality defects on computed tomography at 14 years old. Nasal nitric oxide values were low at 19 nL/min on two occasions, and spirometry showed a forced expiratory volume in 1 second at 104% predicted.

Previously, we demonstrated aberrant splicing by transcript analysis of the c.5778 + 1G > A variant, confirming its pathogenicity by causing an out-of-frame deletion of exons 32 to 35, predicted to lead

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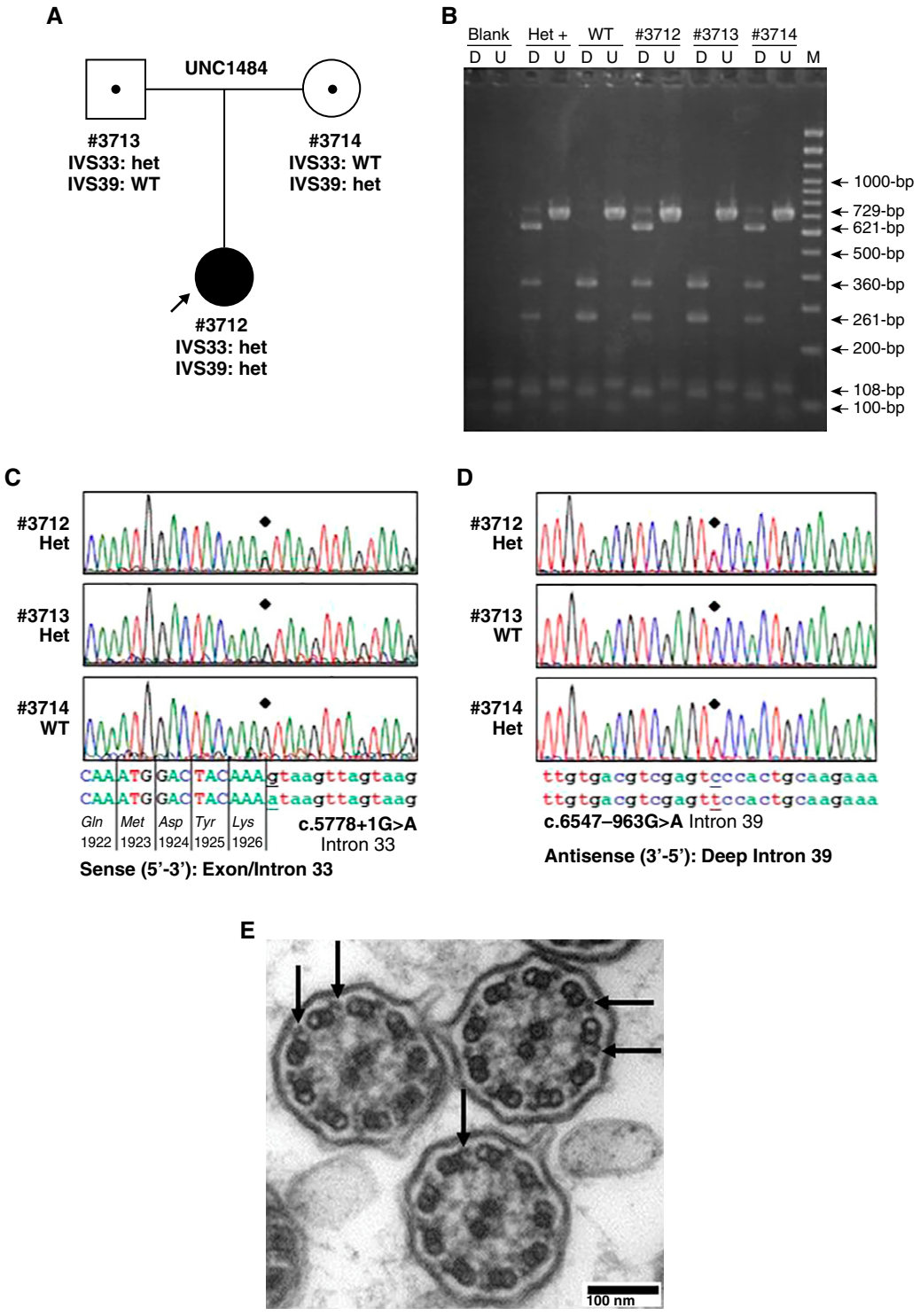

Figure 1. Segregation and location of pathogenic variants in *DNAH11* (dynein axonemal heavy chain 11), ciliary ultrastructure, and computed tomography of the chest for proband #3712. (*A*) Clinical multigene panel by Blueprint Genetics (https://blueprintgenetics.com/tests/panels/nephrology/primaryciliary-dyskinesia-panel/) using next generation sequencing was performed for proband (#3712). A canonical splice site variant c.5778 + 1G > A (p.splice) in intron 33 (IVS33) (https://www.ncbi.nlm.nih.gov/snp/rs72657333) was identified in *DNAH11* (NM_001277115.1). Upon targeted testing, a deep intronic variant c.6547–963G > A (p.splice) in intron 39 (IVS39) (https://www.ncbi.nlm.nih.gov/snp/rs764374746) was identified in the proband. Segregation analysis using Sanger sequencing as well as restriction digestion with *BPU10I* (restriction-enzyme) from an unaffected father (#3713) and mother (#3714) revealed both variants were inherited in trans, consistent with autosomal recessive trait. Males and females are designated by square and circles, respectively. Filled symbol with an arrow shows proband with primary ciliary dyskinesia (PCD) and symbols with a dot within shows carrier status. (*B*) Targeted screening for the deep intron 39 variant

to premature translation termination (p.Val1821Thrfs*8) (6). Aberrant transcript on lymphoblastoid cells has been reported in an 'online supplement' for the c.6547–963G > A variant, causing activation of a cryptic exon of 38-bp within intron 39 (chr7:21,746,318- 21,746,355) (8). The out-of-frame inclusion of the cryptic exon is predicted to cause premature translation termination (p.Ile2183Lysfs*15) (8). The c.6547–963G > A variant is rare and absent from gnomAD, ClinVar, HGMD and other samples in the 100,000 Genomes Project (8).

We report a confirmed case of PCD with a deep intronic, pathogenic variant in a common PCD-related gene, *DNAH11*. This variant was recently reported in one case as likely pathogenic per American College of Medical Genetics guidelines (10) based on its rarity in public variant databases, inheritance in *trans* with another loss-of-function variant, prediction of being deleterious by in-silico method, and transcript analysis (8). Of note, the previously reported intronic variant appeared in trans with a different exonic variant than we report here. In that report, authors identified the non-coding, seemingly pathogenic variant using computational methods and the SpliceAI package (splicing prediction program) that directed follow on transcript analysis, showing this approach to be a superior strategy for discovery and the initial pathogenicity classification of non-coding splice site variants. Despite this advancement, accurate interpretation of genetic variants remains challenging with available guidelines (10) and becomes even more challenging when dealing with non-coding variants. Inclusion of our additional case further supports the pathogenic nature of this variant and suggests other probable PCD cases with monoallelic, disease-causing variants are ideal candidates for whole genome sequencing and computational analysis to investigate "second hits" in non-coding regions of their suspected PCD gene.

Some PCD patients with splice site variants manifest milder disease phenotypes (11, 12). Our case is congruent with this concept, showing excellent lung function and lack of bronchiectasis in adolescence. However, PCD severity from non-coding variants has not been described, and it is unclear if the mild phenotype here is driven by the deep intronic splice variant or in combination with the perhaps 'leaky' canonical splice-donor site variant. Further characterization of these variants at the protein and cellular levels are warranted to explore basic processes and interactions which may shed light on the milder phenotype observed in our patient with this intronic variant.

A similar disease-causing, deep intronic variant has been reported in the *CCDC39* gene (13). *DNAH11* and *CCDC39* are two common PCD-related genes and account for 6–9% and 4–9% of PCD cases, respectively (3). These are larger genes, with 82 exons in *DNAH11* and 20 exons in *CCDC39*. The larger sizes increase the likelihood of harboring disease-causing, exonic variants. One can postulate a similar increase in disease-causing, intronic variants. Following this logic, other large PCD genes, including *DNAH5*, *DNAI1*, *HYDIN*, and *CCDC40* are ideal candidates for further intronic investigations. To our knowledge, no other deep intronic, disease-causing variants have been reported in PCD, a genetically heterogeneous disease, where 20–30% of patients have inconclusive genetic testing. Exploration of noncoding regions will be key to establishing genetic diagnoses in other unsolved PCD cases. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Figure 1. (*Continued*). was performed using c.6547–963 (p.splice) variant induced abrogation of cleavage at a *BPU10I* site. A 729-bp fragment was amplified using M13-tagged forward (5′AGAGGATGGCAGTATATGGAAC-3′) and Reverse (5′-TAGAGACCAGGGAGGTTGCT-3′) primers followed by restriction digestion with *BPU10I*. PCR product from wild-type (WT) alleles cleaves into 3 fragments of 360, 261, and 108-bp, whereas the heterozygous variant alleles shows 4 fragments of 621, 360, 261, and 108-bp that can be visualized by 2% agarose gel. Lanes D, U, and M corresponds to the digested (D), Undigested (U) and 100-bp ladder from Invitrogen (M), respectively. Water blank, heterozygous (Het) positive, and wild-type (WT) controls are shown. Proband (#3712) and unaffected mother (#3714) were heterozygous, while unaffected father (#3713) was wild-type for the deep intron 39 variant. (*C* and *D*) Chromatograms showing genomic DNA sequencing for the wild-type and heterozygous variants in the pedigree for introns 33 and 39. Base sequence, amino-acid sequence, and codon number are shown. Location of the disease-causing variant is shown by a filled diamond and the bases are underlined. (*E*) Normal ciliary ultrastructure with intact inner dynein arms and outer dynein arms (black arrows) on transmission electron microscopy (120,000× magnification).

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