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Rare Genetic Variants in *PARN* Are Associated with Pulmonary Fibrosis in Families

To the Editor:

Rare genetic variation in genes related to telomere biology has been implicated in 10–20% of familial interstitial pneumonia (FIP), the inherited form of idiopathic interstitial pneumonia (1). Recently, heterozygous rare variants (RVs) in the gene encoding polyadenylation-specific RNase deadenylation nuclease (*PARN*) were reported in six unrelated families with pulmonary fibrosis (2), consistent with reports of biallelic *PARN* RVs in children with dyskeratosis congenita (3–5). Subsequently, heterozygous *PARN* RVs were identified in five patients with sporadic idiopathic pulmonary fibrosis (IPF) among 262 patients who underwent whole-exome sequencing (6).

We queried whole-exome sequencing data from genomic DNA obtained from 188 unrelated FIP kindreds (7) for RVs in *PARN,* identified variants with a minor allele frequency <0.001 among Caucasian patients in the Exome Aggregation Consortium database, and confirmed these RVs by Sanger sequencing. Using this approach, we found 13 unique *PARN* RVs in 12/188 (6.4%) unrelated families (Figure 1); seven families (3.7%) had variants predicted to be protein-altering (frameshift, nonsense, splicing, missense; Table 1). In five of these families (2.6% of the cohort), *PARN* RVs identified as likely to be damaging fully cosegregated with disease in individual family members. These *PARN* RVs included one nonsense, one frameshift, one splicing, and two missense variants. The two missense variants, Asn7His and Lys56Ans, are conserved and predicted to affect protein function. For the c.620+5G>A splicing variant, we generated immortalized lymphocytes and performed complementary DNA sequencing; this confirmed that this variant results in alternative splicing, which is likely to affect PARN structure and function.

Surprisingly, in three families, intronic *PARN* variants that did not appear to affect mRNA splicing cosegregated with disease. Complementary DNA sequencing demonstrated that c.178−3C>T does not affect splicing, indicating this is likely benign. Another intronic variant, c.703-11_703-10delAT, is located in an intron near a splice site but is not predicted to alter the canonical splice site or create a cryptic splice site. A third intronic *PARN* variant c.1006−11G>A cosegregated with disease in one family but is not predicted to alter splicing. In this family, however, affected subjects share a novel cosegregating RV in the gene telomerase reverse transcriptase (Thr839Lys). Although available evidence suggests these intronic RVs are benign, their cosegregation with disease and association with otherwise unexplained short telomeres in affected individuals raises the possibility that these intronic variants have effects on PARN expression or other regulatory mechanisms.

In the remaining four families, *PARN* RVs did not fully segregate with disease. In two of these families, there was a family history of IPF through both parental lineages, making it possible that affected individuals inherited a different genetic risk factor through each parental line. In one family, an intronic *PARN* RV that could affect splicing (c.245+75_245+77delCCC) was identified in a patient with IPF, whereas the other affected individuals shared a frameshift variant (Phe418PhefsX6). In a different family, an affected individual with short peripheral blood mononuclear cell telomeres carried a missense RV (Ser498Asn) that is predicted to be deleterious (PolyPhen2 0.996) (8), but the RV was not identified in the other family member with disease.

We measured peripheral blood mononuclear cell telomere length in all affected individuals from these families from whom sufficient DNA was available and found that all had telomere shortening adjusted for age (Table 1). Thirteen of the 18 subjects tested (72%) had telomere length below the 10th percentile, whereas the others ranged from the 12th to the 22nd percentile. All these families were of Caucasian ancestry, and 62% of affected subjects were men. The median age at diagnosis was 60 years (range, 42–82 years), slightly younger than the median age of onset in our entire cohort of patients with FIP (66 years) (7). Forty-three percent of affected subjects had a history of cigarette smoking. Baseline FVC was 68.5% (±17.7%, SD) predicted,

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Table 1. *PARN* Rare Variants Identified by Whole-Exome Sequencing

Variant Type	Variant (Protein)	Variant (DNA)	Splicing Effect	ExAC DB Frequency	PP2	Affected Carrier Telomere %	Segregates with Disease
Loss of function variants							
Frameshift	p.Thr296SerfsX14	c.887_888delCA	NA	None	NA	2%, 3%	Yes
Splice	NA	c.620+5G>A	Yes	None	NA	1%	Yes
Nonsense	Glu189Stop	c.565G>T	NA	None	NA	1%, 1%, 12%	Yes
Frameshift	p.Phe418PhefsX6	c.1251delT	NA	None	NA	15%	No*
Variants of uncertain significance							
Missense	Asn7His	c.19A>C	Unknown	None	1	3%	Yes
Missense	Lys56Asn	c.168G>C	NA	G=1/C=66,604	0.667	5%, 5%	Yes
Intron	NA	c.178-3C>T	No	None	NA	7%, 9%	Yes
Intron	NA	c.703-11_703-10delAT	Unknown	None	NA	1%, 2%	Yes
Intron	NA	c.245+75_245+77delCCC	Unknown	NA	NA	15%	No*
Synonymous	Ala153Ala	c.459G>C	Unknown	None	NA	21%, 22%	No†
Intron	NA	c.840+6T>C	Unknown	G=33/A=66,636	NA	1%	No‡
Intron	NA	c.1006-11G>A	Unknown	G=3/A=65,604	NA	1%, 2%	Yes¶
Missense	Ser498Asn	c.1493G>A	NA	G=20/A=60,728	0.996	1%	No

Definition of abbreviations: ExAC DB = Exome Aggregation Consortium database; NA = not applicable; *PARN* = polyadenylation-specific RNase deadenylation nuclease; PP2 = PolyPhen2; *TERT* = telomerase reverse transcriptase.

*p.Phe418PhefsX6 was not detected in one affected participant; however, this subject carried an intronic variant of uncertain significance, c.245+75_245+77delCCC.

†One subject did not carry c.459G but had a family history of idiopathic pulmonary fibrosis in both parental lines.

‡Two subjects did not carry c.840+6T>C; however, they had a family history of idiopathic pulmonary fibrosis in both parental lineages.

¶This family also has a novel segregating *TERT* variant Thr839Lys.

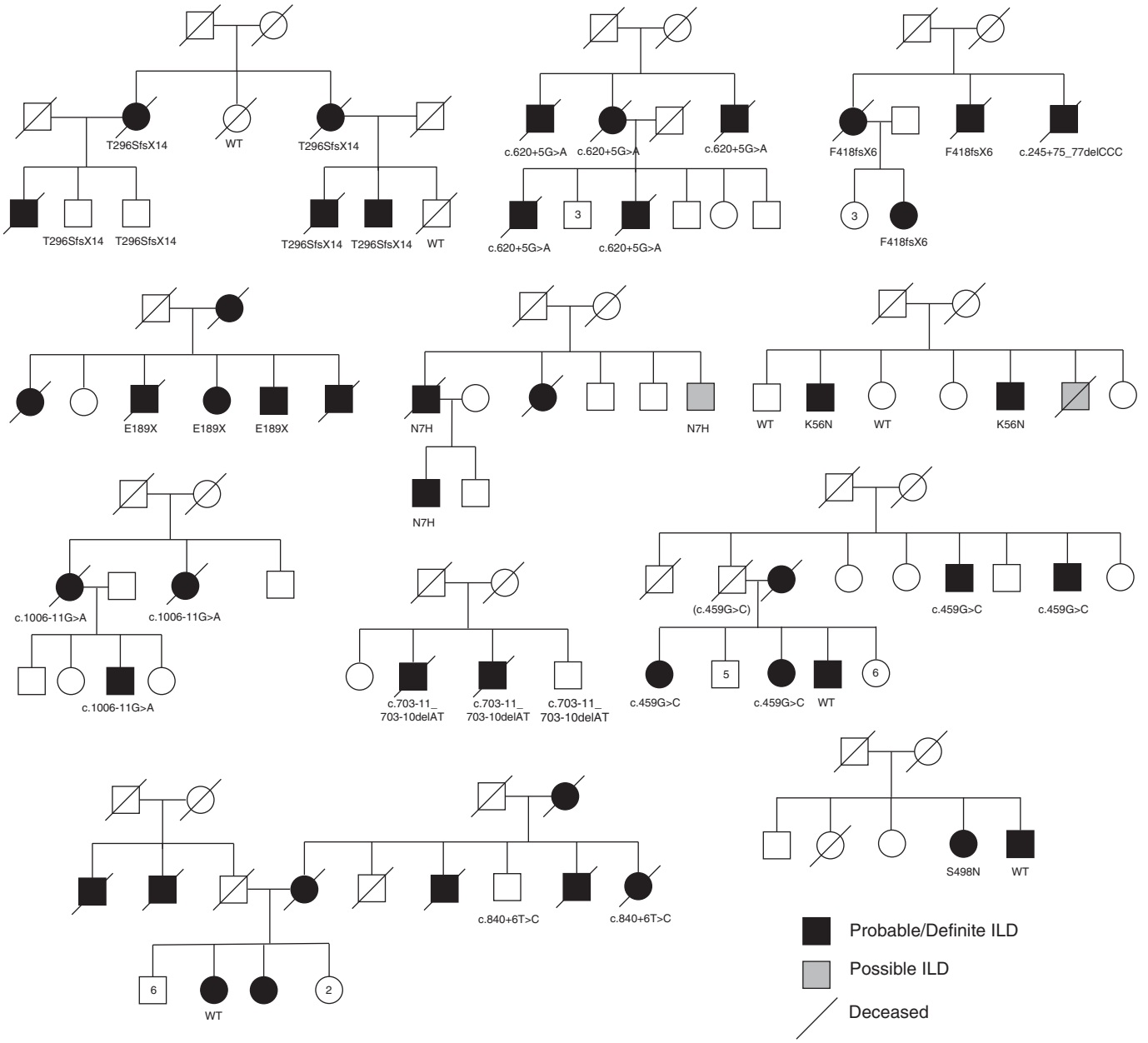

Figure 1. Pedigrees of familial interstitial pneumonia kindreds with polyadenylation-specific RNase deadenylation nuclease (*PARN*) variants. Variant information is denoted below individuals who had DNA available for sequencing. Numbers inside pedigree symbols indicate the number of other siblings of the same sex and affected status. ILD = interstitial lung disease; WT = wild type.

and diffusing capacity for carbon monoxide was 66.5% (±24.0%). Among individuals for whom high-resolution computed tomography was available for review, all had possible or definite usual interstitial pneumonia based on American Thoracic Society guidelines (9).

Recent work has indicated that PARN polyadenylates the 3′ end of telomerase RNA component (known as *TERC* or *hTR*), which serves as the template for telomerase reverse transcriptase–mediated telomere replication. Presumably, *PARN* mutations destabilize hTR levels (10) and lead to reduced telomerase activity through a haploinsufficiency mechanism similar to dyskerin (*DKC1*) mutations (11); further investigation will be needed to determine whether *PARN* plays other roles in telomere biology. Exciting recent work suggests inhibiting RNA-decay mechanisms may reverse these cellular phenotypes, suggesting a possible novel approach to personalizing therapy in pulmonary fibrosis (12, 13).

Our data provide independent confirmation of genetic variation in *PARN* as an important influence on FIP risk. In addition, these findings underscore the genetic complexity and heterogeneity of FIP. Given this complexity and the difficulty in assigning causality to variants of uncertain significance in affected individuals, our current practice is, after genetic counseling, to perform clinical genetic testing for *PARN* or other telomere pathway genes along with telomere length measurement, which provides some evidence regarding the functional importance of telomerase

pathway genetic variants (1). However, as illustrated by the families reported here, even with the combination of telomere length measurement and genetic testing, assignment of disease risk to individual RVs may be difficult. As the spectrum of genetic risk for familial and sporadic IPF is expanded, we anticipate that enhanced understanding of the complex genetic influences underlying this disease will improve our ability to use genetic information in the care of these patients. ■

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Cardiac Morphometry on Computed Tomography and Exacerbation Reduction with β-Blocker Therapy in Chronic Obstructive Pulmonary Disease

To the Editor:

Chronic obstructive pulmonary disease (COPD) is associated with cardiovascular disease (1), and a subset of COPD exacerbations may be the result of overt or subclinical cardiovascular disease (1). We, and others, have shown that the use of cardiac function modulating β-blockers is associated with substantially lower rates of exacerbations (2). COPD is associated with functional and structural

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