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Primary Ciliary Dyskinesia-Causing Mutations in Amish and Mennonite Communities

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Abstract

Objective—To determine whether individuals with primary ciliary dyskinesia (PCD) from unrelated Amish and Mennonite families harbor a single and unique founder mutation.

Study design—Subjects from Amish and Mennonite communities in several states were enrolled in the study. All subjects were clinically characterized, and nasal nitric oxide levels were measured. Nasal epithelial scrapings were collected from several subjects for ciliary ultrastructural analyses. DNA was isolated from patients with PCD and their unaffected first- and second-degree relatives. Genome-wide homozygosity mapping, linkage analyses, targeted mutation analyses, and exome sequencing were performed.

Results—All subjects from Old-Order Amish communities from Pennsylvania were homozygous for a nonsense mutant *DNAH5* allele, c.4348C>T (p.Q1450X). Two affected siblings from an unrelated Mennonite family in Arkansas were homozygous for the same nonsense *DNAH5* mutation. Children with PCD from an Amish family from Wisconsin had biallelic *DNAH5* mutations, c.4348C>T (p.Q1450X) and c.10815delT (p.P3606HfsX23), and mutations in other genes associated with PCD were also identified in this community.

Conclusion—The Amish and Mennonite subjects from geographically dispersed and socially isolated communities had the same founder *DNAH5* mutation, owing to the common heritage of these populations. However, disease-causing mutations in other PCD-associated genes were also found in affected individuals in these communities, illustrating the genetic heterogeneity in this consanguineous population.

The first human genetic disorder associated with ciliary dysfunction, primary ciliary dyskinesia (PCD) usually has an autosomal recessive mode of inheritance that leads to functional and, in some cases, ultrastructural abnormalities of the cilia. Impaired ciliary motility results in retention of inhaled particles and bacteria in the lung, paranasal sinuses, and middle ear, which manifest as chronic bronchitis, pansinusitis, and suppurative otitis media.^{1–4} Dysfunction of cilia outside of the sinopulmonary tract may cause male infertility

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owing to impaired movement of spermatozoa, and can play a role in laterality defects, such as situs inversus totalis, related to motility defects of nodal cilia.¹⁻⁴

The variable clinical presentation of PCD is likely related to the genetic heterogeneity of this disease, which reflects the complexity of the ciliary structure. Cilia are composed of hundreds of proteins, and their chemical and physical interactions are required for motility to generate an effective and synchronous waveform.⁵⁻⁷ Mutations in any of these cilia proteins could potentially result in functional impairment and a spectrum of clinical manifestations. Genetic causes of PCD have been identified by sequencing human orthologs of genes discovered by screening for ciliary dysfunction in model organisms, which have provided insight into genotype-phenotype relationships in the human cilium. Using such models, mutations in several different genes, including *DNAI1*⁸⁻¹⁰ and *DNAH5*,^{11,12} have been shown to cause PCD; these mutations account for disease in roughly half of all affected individuals.

The Old-Order Amish and Mennonite populations in North America have a common social heritage, descending from Swiss Anabaptist immigrants who emigrated seeking religious tolerance in the eighteenth century. Starting from initial settlements in Pennsylvania, these close-knit agrarian communities have spread across North America. Settlements maintain strict adherence to religious customs, and because members rarely marry outside the church, consanguinity is common. Families live in small endogamous communities that have different allele frequencies and genetic diseases due to unique founders, resulting in closed populations with a limited gene pool and an increased incidence of certain heritable conditions.¹³⁻¹⁶ In previous studies of Old Order Amish and Amish-Mennonite communities in the US, we discovered individuals with clinical manifestations consistent with PCD from several unrelated communities. Owing to consanguinity present in these geographically dispersed populations, we investigated the possibility that these communities harbor unique founder mutations.

Methods

Subjects from Amish communities in the midwest with neonatal respiratory distress, situs inversus totalis, chronic pneumonia, or rhinosinusitis were recruited to participate in the study, which has been described previously.¹⁷ Two probands from an Old-Order Amish community in Pennsylvania were enrolled and evaluated in a cross-sectional study supported by the Genetic Diseases of Mucociliary Clearance Consortium, and other family members with classic clinical features (Table I) were studied at the Clinic for Special Children. The Mennonite subjects from Arkansas were also enrolled in studies administered by the Genetic Diseases of Mucociliary Clearance Consortium and studied at the University of North Carolina at Chapel Hill. Evaluations included detailed clinical and family histories and physical examination. Nasal nitric oxide (NO) levels were measured in all subjects using established techniques with a spirometer (model CLD 88; Eco Medics, Duernten, Switzerland). Spirometry was performed on subjects aged 5 years. Sputum or oropharyngeal swabs were collected for microbiological studies. Nasal epithelial scrapings from inferior nasal turbinates were collected from several subjects for ultrastructural analysis of ciliary axonemes,^{3,18} which revealed dynein arm defects. Blood or buccal cell samples were collected for genetic analysis; DNA was extracted using commercially available kits.¹⁷ The diagnosis of PCD was based on the presence of typical clinical features, reduced nasal NO levels, and ultrastructural defects or disease-causing mutations of known genes. The relevant Institutional Review Boards approved the studies, and signed informed consent was obtained from all subjects and family members.

Specimens were collected for DNA isolation from subjects with PCD and unaffected first- and second-degree relatives with known consanguinity from Plain communities from Arkansas, Illinois, Missouri, Pennsylvania, and Wisconsin. In addition, DNA from unrelated Amish and Mennonite children with other genetic conditions without symptoms consistent with PCD were analyzed, to determine the relative frequency of *DNAH5* mutations in Pennsylvania settlements.

DNA was extracted from blood or buccal swab specimens, and whole genome amplification was performed when necessary to provide adequate genetic material for further analysis. Genome-wide homozygosity mapping, using high-density single nucleotide polymorphism arrays, was performed following established protocols.¹⁹ Targeted mutation analyses were conducted by sequencing 9 exons of *DNAH5* and *DNAI1* using a clinical genetic panel developed at the University of North Carolina at Chapel Hill, which was developed based on results from large studies that showed at least 1 mutation present in the selected exons and splice sites in *DNAI1* (exons 1, 13, 16, and 17) and *DNAH5* (exons 34, 50, 63, 76, and 77) in most affected individuals in whom PCD was due to the mutations in those genes.⁸⁻¹² This panel reduced DNA sequencing from a total of 99 exons. When only a single mutation was detected, all of the coding exons and splice sites were interrogated for the pertinent gene to identify a possible second mutant allele. Moreover, if biallelic mutations were not detected, at least 1 affected individual from that cohort was sequenced for all coding exons and splice sites for *DNAI1* and *DNAH5*, to detect any mutations in exons not covered initially.²⁰

To investigate linkage with 9 candidate genes known to be involved in outer dynein arm structure (*DNAI1*, *DNAH5*, *DNAH11*, *DNAH9*, *DNAI2*, *DNAL4*, *DNCL1*, *DNL2A*, and *TCTEL1*), affected individuals and family members from Midwestern Amish-Mennonite communities were genotyped for polymorphic microsatellites within and flanking these genes. Haplotypes were reconstructed such that recombinants could be visualized on pedigrees.

Results

All of the subjects studied had classic features, and ultrastructural examination of the ciliary axoneme revealed dynein arm defects in several affected individuals consistent with a diagnosis of PCD (Table I). Identification of multiple members from several Amish families from central Pennsylvania facilitated genome-wide homozygosity mapping, using high-density single nucleotide polymorphism arrays. These analyses localized the disease locus to chromosome 5 in a region containing a known PCD-causing gene, *DNAH5*. Sequencing identified a nonsense mutation in exon 27, c.4348C>T (p.Q1450X), that was previously reported in a patient of Swiss descent.²¹ All of the affected individuals from this cohort were homozygous for this nonsense mutation (Figure, A; III4, V1, V4, V6, V7, V8, V9, and V10). This variant was also found in 2 affected siblings from an apparently nonconsanguineous Mennonite family in Arkansas (Figure, B; II2 and II3). Exomic analyses of 102 unrelated children without PCD from Amish and Mennonite families in Pennsylvania identified 18 missense or nonsense variants in *DNAH5*,^{11,12,21} including 1 subject heterozygous for *DNAH5* c.4348C>T (p.Q1450X) (Table II).

Resequencing of the *DNAH5* coding region and flanking introns from subjects and family members from a large Midwestern Amish-Mennonite community revealed compound heterozygous *DNAH5* mutations in 2 affected siblings: c.10815delT (p.P3606HfsX23) and c.4348C>T (p.Q1450X) (Figure, C; VI4 and VI5); however, several other family members with PCD did not have *DNAH5* mutations. Genetic testing showed that 5 subjects (Figure, C; V8, VII0, VII1, VII2, and VII3) were heterozygous for the founder *DNAI1* mutation, g.IVS1+2_3insT (c.48+2_3insT). Analyses of microsatellite genotype data confirmed that

other affected individuals were discordant for microsatellite marker alleles at known PCD loci, including those associated with *DNAH5* and *DNAI1*, indicating an unidentified PCD-causing mutation in a novel gene. Subsequently, whole-exome sequencing revealed an autosomal recessive, nonsynonymous missense mutation in *HEATR2* (p.L795P), an uncharacterized gene not previously associated with ciliary assembly or function.²² Of note, 3 affected children were homozygous for the *HEATR2* mutation and heterozygous for the *DNAI1* mutation (VI11, VII2, and VII3), further illustrating the complex genetic basis of PCD in this community.

Discussion

PCD is a genetically heterogeneous, autosomal recessive disease resulting from impaired ciliary function. The estimated incidence is 1 in 15 000–30 000 births,^{23,24} with an estimated prevalence as high as 5% in children that have recurrent respiratory infections. Investigators have identified 15 different genes that when mutated produce unambiguous clinical phenotypes and in most cases ultrastructural defects of the ciliary axoneme.^{4,19,22,25,26}

Arising from an Anabaptist movement, the Swiss Brethren, the endogamous Plain communities of North America present unique opportunities to better understand the genetic basis of PCD and identify new candidate genes. Relative isolation, detailed record keeping, and consanguineous marriages make Amish families ideal for genetic studies. We found a high prevalence of PCD in Amish settlements, based on classic clinical history, reduced nasal NO levels, and ultrastructural defects of the cilia. Many individuals harbored axonemal γ -dynein heavy chain *DNAH5* mutations; affected individuals in Pennsylvania Amish communities and 2 subjects from a Mennonite family in Arkansas were homozygous for the nonsense c.4348C>T (p.Q1450X) mutation. Two Amish subjects from the midwestern US had biallelic mutations of *DNAH5*, specifically c.4348C>T (p.Q1450X) and frameshift c.10815dT (p.P3606HfsX23) mutations.

Homologous to *HC γ* in the biflagellated alga *Chlamydomonas reinhardtii*, *DNAH5* is one of the first genes to have been implicated with disease.^{11,12} Since the discovery of *DNAH5* and its involvement in PCD, more than 60 disease-causing mutations in the gene have been identified. Previously, investigators from Europe and the North America reported that 53% of patients with PCD and known outer dynein arm defects had mutations clustered in 6 exons of *DNAH5*,¹¹ and *DNAH5* is the most common gene linked to PCD with outer dynein arm defects,¹¹ although the actual frequency of these mutations may be lower than reported.²⁰

In addition, we previously shown that some affected individuals from the midwestern Amish families were heterozygous for a founder mutation, g.IVS1+2_3insT (c.48+2_3insT) in *DNAI1* (Figure, C), the human ortholog of *Chlamydomonas reinhardtii IC78* and the first gene associated with PCD identified using the candidate gene approach.¹⁷ This variant was not disease-causing in these families, because microsatellite marker analysis excluded *DNAI1*. Moreover, a second mutation was not identified after whole-exome sequencing.¹⁷

Even though we found PCD-causing mutations in *DNAI1* and *DNAH5* in these Anabaptist communities, most subjects in the midwestern Amish families had an unidentified mutation in a novel gene. Whole-exome sequencing revealed a single autosomal recessive missense mutation in *HEATR2* (p.L795P). Airway epithelial cells isolated from individuals with PCD had markedly reduced cytoplasmic HEATR2 levels, absent dynein arms, and virtually immotile cilia. Recent analyses indicated a role in dynein arm transport and assembly.²²

In conclusion, we have found that *DNAH5* mutations are common to individuals with PCD from socially and geographically isolated Amish and Mennonite communities in the US. In

addition, we have identified a known *DNAI1* splice mutation and novel missense mutation in *HEATR2* in these families. Taken together, a panel of these mutations could be a useful diagnostic tool for such patients who present with clinical features consistent with PCD.

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Glossary

NO	Nitric oxide
PCD	Primary ciliary dyskinesia

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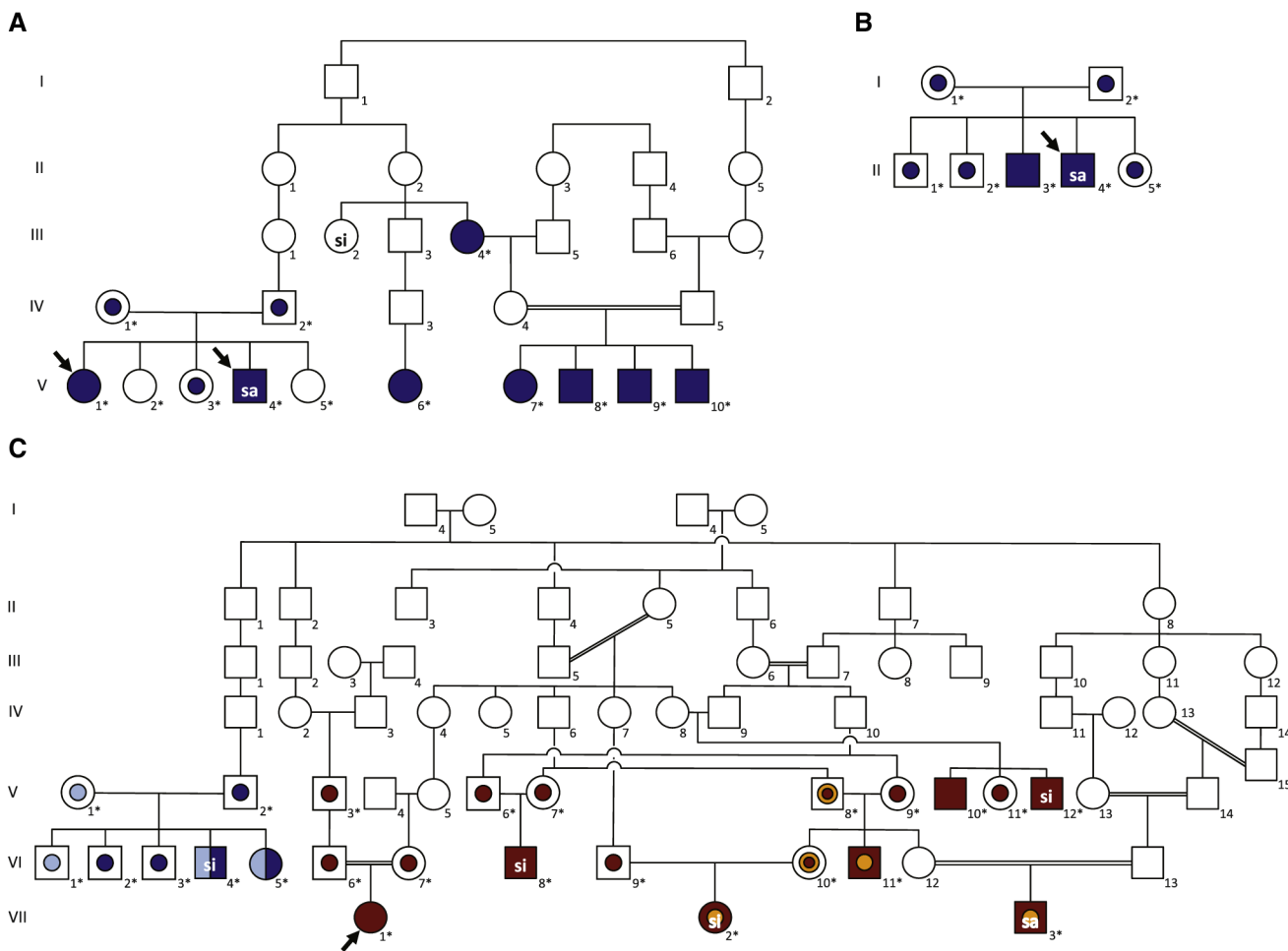


Figure. Family pedigrees and genetic analyses. **A**, Consanguineous kindred with related nuclear families in Amish communities from Pennsylvania. **B**, Mennonite family from Arkansas. **C**, Amish-Mennonite family with children with PCD from several midwestern states, in which affected individuals had 2 heteroallelic *DNAH5* mutations. *Solid symbols* indicate affected individuals; *central dots*, heterozygous individuals; *dark-blue symbols*, c.4348C>T (p.Q1450X) *DNAH5* mutation; *light-blue symbols*, c.10815delT (p.P3606HfsX23) *DNAH5* mutation; *yellow symbols*, *DNAIL1* (g.IVS1+2_3insT) mutation; *red symbols*, *HEATR2* (p.L795P) mutation. *Arrows* specify the probands. *Asterisks* indicate subjects who underwent genetic testing. *si*, situs inversus totalis; *sa*, situs ambiguus

Table 1

Clinical characteristics and diagnostic testing of Amish and Mennonite subjects with PCD and *DNAH5* mutant alleles

Patient	Age	Sex	Clinical manifestations	Laterality	Ultrastructural defect	Nasal NO, nL/min*	<i>DNAH5</i> mutation
Pennsylvania							
1	14 y	Female	RS, OM, HL, PC, BR	SS	ODA	15.6	4348C>T/4348C>T
2	7 y	Male	NRD, CHD, RS, OM, HL, PC, BR	SA	ODA	5.7	4348C>T/4348C>T
3	10 m	Male	OM	SS	ND	68.0	4348C>T/4348C>T
4	63 y	Female	RS, PC, BR	SS	ND	14.3	4348C>T/4348C>T
5	9 y	Female	NRD, OM, RS, PC	SS	ND	31.5	4348C>T/4348C>T
6	6 y	Male	NRD, OM, HL, RS, PC	SS	ND	40.5	4348C>T/4348C>T
7	11 y	Male	NRD, OM, HL, RS, PC	SS	ND	26.5	4348C>T/4348C>T
8	13 y	Female	NRD, OM, HL, RS, PC	SS	ND	26.7	4348C>T/4348C>T
Arkansas							
1	13 y	Male	RDS, CHD, OM, HL, RS, PC	SA	ND	8.9	4348C>T/4348C>T
2	18 y	Male	RDS, OM, HL, RS, PC	SS	ND	9.2	4348C>T/4348C>T
Wisconsin							
1	3 m	Male	NRD, RS, OM, PC	SS	ODA	8.6	10815dT/4348C>T
2	2 y	Female	NRD, RS, OM, HL, PC	SI	ODA	8.1	10815dT/4348C>T

BR, bronchiectasis; CHD, congenital heart disease; HL, hearing loss; ND, not done; NRD, neonatal respiratory distress or hypoxemia; ODA, outer dynein arm; OM, chronic or recurrent otitis media; PC, daily productive cough; RS, rhinosinusitis; SA, situs ambiguus; SI, situs inversus totalis; SS, situs solitus.

DNAH5 mutations: exon 27 c.4348C>T (p.Q1450X) and exon 63 c.10815delT (p.P3606HfsX23).

* Normal nasal NO level, >100 nL/min.

Exomic analysis revealing *DNAH5* sequence variants on chromosome 5 in 102 unaffected individuals from Pennsylvania Amish communities

Table II

Position	Identification no.	Reference	Alternate	Protein change	Variant	Mutation class
13701536	rs3734110	T	C	p.I4450V	Novel	Missense
13717471	rs2277046	T	C	p.T4220A	Novel	Missense
13719089	rs30168	G	A	p.A4134V	Novel	Missense
13719111	rs148696723	G	A	p.R4127C	Novel	Missense
13737444	rs17263496	G	A	p.T3791I	Novel	Missense
13754379	-	C	A	p.L3496F	Novel	Missense
13788886	rs10513155	C	A	p.L2862F	Novel	Missense
13811775	rs10078391	T	C	p.Q2463R	Novel	Missense
13817740	-	A	C	p.F2302C	Novel	Missense
13865784	CM091933*	G	A	p.Q1450X	Reported	Nonsense
13866375	rs200516414	C	T	p.S1357N	Novel	Missense
13871757	rs141168110	G	T	p.Q1172K	Novel	Missense
13871811	-	C	T	p.E1154K	Novel	Missense
13894894	rs4701997	T	A	p.I766L	Novel	Missense
13902220	rs1530498	T	C	p.T558A	Novel	Missense
13902245	rs139160176	G	C	p.N549K	Novel	Missense
13931340	rs1530496	C	T	p.G24E	Novel	Missense
13944512	rs339445	A	C	p.H12Q	Novel	Missense

dbSNP, Single Nucleotide Polymorphism Database; *HGMDB*, Human Gene Mutation Database; *NCBI*, National Center for Biotechnology Information.

Annotation and literature search: NCBI dbSNP, Ensembl Genome Browser (www.ensembl.org), and HGMD Public (www.hgmd.cf.ac.uk/ac/index.php).

* Denotes HGMD identification number; all other identifiers are from NCBI dbSNP (build 137).