Original Article

Next-generation sequencing for identifying genetic mutations in adults with bronchiectasis

Wei-Jie Guan[1,2#], Jia-Cheng Li[3#], Fang Liu[3], Jian Zhou[3], Ya-Ping Liu[3], Chao Ling[4], Yong-Hua Gao[5], Hui-Min Li[1], Jing-Jing Yuan[1], Yan Huang[1], Chun-Lan Chen[1], Rong-Chang Chen[1], Xue Zhang[3], Nan-Shan Zhong[1]

[1]State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou 510120, China; [2]Sino-French Hoffmann Institute, Guangzhou Medical University, Guangzhou 510120, China; [3]McKusick-Zhang Center for Genetic Medicine, State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100005, China; [4]Laboratory of Clinical Genetics, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China; [5]Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

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[#]These authors contributed equally to this work.

Correspondence to: Prof. Nan-Shan Zhong, MD. State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Health, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, 151 Yanjiang Road, Guangzhou 510120, China. Email: nanshan@vip.163.com; Prof. Xue Zhang, PhD. McKusick-Zhang Center for Genetic Medicine, State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 5, Dong Dan San Tiao, Beijing 100005, China. Email: xuezhang@pumc.edu.cn.

Background: Defective airway host-defense (e.g., altered mucus properties, ciliary defects) contributes to the pathogenesis of bronchiectasis. This study aims to determine whether genetic mutations associated with defective airway host-defense are implicated in the pathogenesis of bronchiectasis.

Methods: Based on the systematic screening of 32 frequently reported bronchiectasis-associated genes, we performed next-generation sequencing (NGS) on peripheral blood samples from 192 bronchiectasis patients and 100 healthy subjects. The variant distribution frequency and pathogenicity of mutations were analyzed.

Results: We identified 162 rare variants in 192 bronchiectasis patients, and 85 rare variants among 100 healthy subjects. Among bronchiectasis patients, 25 (15.4%), 117 (72.2%) and 18 (11.1%) rare variants were associated with cystic fibrosis transmembrane receptor (*CFTR*), epithelial sodium channel, and primary ciliary dyskinesia genes, respectively. Biallelic *CFTR* variants were detected in four bronchiectasis patients but none of the healthy subjects. Carriers of homozygous p.M470 plus at least one *CFTR* rare variant were detected in 6.3% of bronchiectasis patients (n=12) and in 1.0% of healthy subjects (n=1, P=0.039). Twenty-six patients (16 with idiopathic and 6 with post-infectious bronchiectasis) harbored biallelic variants. Bronchiectasis patients with biallelic *DNAH5* variants, or biallelic *CFTR* variants plus an epithelial sodium channel variant, tended to have greater disease severity.

Conclusions: Genetic mutations leading to impaired host-defense might have implicated in the pathogenesis of bronchiectasis. Genetic screening may be a useful tool for unraveling the underlying causes of bronchiectasis, and offers molecular information which is complementary to conventional etiologic assessment for bronchiectasis.

Keywords: Bronchiectasis; mutation; cystic fibrosis transmembrane conductance regulator; primary ciliary dyskinesia; next-generation sequencing (NGS)

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Introduction

Bronchiectasis is characterized by irreversible bronchial dilatation resulting from airway infection and inflammation (1). Various etiologies have been attributable to bronchiectasis (2-4), but whether genetic mutations are underlying causes is unclear. Certain mutations (e.g., *RSPH1*, *RSPH4A*, *RSPH9* and *DNAI1*) related to primary ciliary dyskinesia (PCD) (5-8), immunodeficiency (9), and cystic fibrosis (CF) (10,11) have been implicated in the pathogenesis of bronchiectasis. However, previous studies were limited by relatively small sample sizes (7,9-11), selective mutation analysis (5-9,11) and children-predominant population (5,6), which limited the generalizability of findings (12).

Next-generation sequencing (NGS) with Ion Torrent PGM™ platform (Life Technologies Inc., USA) has fueled the testing of various diseases (10). Compared with Sanger sequencing, NGS is simpler and convenient, has higher detection accuracy and faster responses at lower cost. NGS confers a greater likelihood for identifying novel mutations (13-16), which might unravel how mutations are implicated in bronchiectasis.

Airway epithelium plays pivotal roles in maintaining host-defense to pathogen invasion. Ciliary defects associated with PCD and cystic fibrosis transmembrane conductance regulator (*CFTR*) disorders have been linked to bronchiectasis pathogenesis. Defects of *RSPH1*, *RSPH9* (17) or *DNAH11* genes (18) could have resulted in bronchiectasis due to impaired ciliary ultrastructure and/or beating.

In view that pathogenic mutations associated with defective airway host-defense predispose to bronchiectasis, we compared mutation frequency between bronchiectasis patients and healthy subjects in Chinese Han ethnicity, identified pathogenic mutations, and determined the clinical characteristics of bronchiectasis with biallelic mutations.

Methods

Subjects

Adults with bronchiectasis, diagnosed with chest high-resolution computed tomography (HRCT), were recruited from The First Affiliated Hospital of Guangzhou Medical University between September 2012 and 2015. Cystic fibrosis was not routinely screened because of extremely low prevalence in China. Healthy subjects with normal chest X-ray and no prior history or symptoms of chronic respiratory diseases (including asthma, chronic obstructive pulmonary disease, etc.) whose data were anonymized before blood collection were enrolled from our health check-up center. None of the subjects had malignancy, severe systemic disorders (e.g., stroke), or upper airway infection or antibiotic use within 4 weeks. Ethics approval was obtained from Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University. Written informed consent was obtained.

Clinical assessments

Venous blood was withdrawn, followed by gradient centrifugation in heparinized tubes. Genomic DNA was extracted using commercialized kits (Qiagen, Valencia, CA).

For bronchiectasis patients, clinical investigations included etiology (4), chest HRCT, spirometry (19) and sputum bacteriology (20). Chest HRCT was assessed using the modified Reiff score (21). Fresh sputum was immediately sent for microbiology assessment. We calculated Bronchiectasis Severity Index (BSI) to determine disease severity (22) (See online Supplement text).

Mutation screening

Mutations of multiple genes have been described as a cause of bronchiectasis (23-25). Thirty-two clinically important candidate genes of cystic fibrosis (*CFTR*, *SCNN1A*, *SCNN1B* and *SCNN1G*), PCD (*CCDC103*, *CCDC114*, *CCDC164*, *CCDC39*, *CCDC40*, *DNAAF1*, *DNAAF2*, *DNAAF3*, *DNAH11*, *DNAH5*, *DNAI1*, *DNAI2*, *DNAL1*, *HEATR2*, *LRRC6*, *OFD1*, *RPGR*, *RSPH4A*, *RSPH9*, and *NME8*) and other lung diseases (*EFEMP2*, *ELN*, *FBLN5*, *FLCN*, *LTBP4*, *SERPINA1*, *ATP7A* and *SLC34A2*) were screened in our study (see the detailed panel in *Tables S1-S3* in Online Supplement text). Data analysis was performed with the hypothesis that bronchiectasis might be a single-gene disorder. The conservation and pathogenicity of the mutations were also determined (*Figure 1*).

In this study, sequencing libraries were constructed using 10 ng of the genomic deoxyribonucleic acid (DNA). The DNA was amplified using a custom-designed Ion AmpliSeq™ Panel (32 genes) (Life Technologies, Grand Island, NY, USA) and barcoded by using the Ion Xpress Fragment Library Kit and Ion Xpress™ Barcode Adapters (Life Technologies, USA). The DNA libraries were then amplified with the OneTouch Template 200 Kit on the Ion One Touch™ 2 (Life Technologies, USA) and enriched on the Ion OneTouch™ ES. The enriched libraries were sequenced on an Ion Torrent PGM platform (Life Technologies Inc., USA) after annealing the sequencing primer, binding with the Ion PGM™ Sequencing Polymerase and loading on the Ion 318™ v2 Chip according to the manufacturer's protocols. Software including Torrent Suite™ 4.0 (Life Technologies Inc., USA) and wANNOVAR (web ANNOVAR) were applied to perform bioinformatic analysis to annotate the genetic variants in terms of disease phenotypes. Finally, all likely pathogenic variants were confirmed by Sanger sequencing.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Ill, USA). We presented numerical data as mean ± standard deviation or median (interquartile range), and categorical data as number (percentage). Independent *t*-test or Mann-Whitney test was employed for two-group comparisons. Chi-square test was performed to compare rates for two groups. $P<0.05$ was deemed statistically significant for all analyses unless otherwise stated.

Results

Patient characteristics

Of 316 subjects screened, 24 withdrew consent. Data from 192 bronchiectasis patients and 100 healthy subjects were analyzed (*Figure 2*).

Our cohort consisted of mainly middle-aged adults with a female predominance. Twenty-two (11.5%) and 11 patients (5.7%) reported a family history of chronic respiratory diseases and bronchiectasis, respectively. The dominant etiology was idiopathic (31.8%), followed by post-infectious (27.6%) (*Table 1*).

Panel performance of candidate genes

Sequencing of 32 genes (see *Table S3* in Online Supplement text) yielded a mean depth of ~200× and coverage of 99.3% (*Figure S1*). Exons with low (<20-fold) or no coverage were subject to Sanger sequencing to achieve 100% coverage. Pathogenic and rare mutations of unknown significance were confirmed with Sanger sequencing.

We identified 162 and 85 rare variants in bronchiectasis patients and healthy subjects, respectively. In bronchiectasis, 25 (15.4%), 117 (72.2%) and 18 (11.1%) rare variants were associated with *CFTR*/epithelial sodium channel (*ENaC*), PCD and other relevant genes, respectively (see *Tables S4*,*S5* in Online Supplement text).

CFTR and ENaC mutations

We examined *CFTR*, *SCNN1A*, *SCNN1B* and *SCNN1G* mutations via screening the published pathogenic mutations from databases, followed by identification with single nucleotide polymorphism frequency <0.1% and other novel mutations. Pathogenicity was predicted with the software Sift (http://sift.jcvi.org) and Polyphen2 (http://genetics.bwh.harvard.edu/pph2/).

Overall, 49 bronchiectasis patients (25.5%) and 26 healthy subjects (26.0%) harbored at least one rare *CFTR* variant, respectively (see *Tables S4*,*S5* in Online Supplement text). Forty-three bronchiectasis patients (22.4%) harbored 15 *CFTR* variants, whereas 6 patients (3.1%) harbored six *SCNN1A* and *SCNN1B* variants. Twenty-five healthy subjects (25.0%) harbored 13 *CFTR* variants, whereas 1 (1.0%) harbored *SCNN1B* variant. We identified five novel variants: c.1252C>A(p.P418T), c.205G>A(p.D69N) and c.953T>G(p.I318S) for *SCNN1A*, and c.3592G>A(p.V1198M) and c.4262T>A(p.V1421E) for *CFTR*.

Eighteen bronchiectasis patients (9.4%) harbored c.1666A>G(p.I556V) in *CFTR* variants (exon 12). No significant differences in c.1666A>G frequency were found compared with healthy subjects (9.0%, P=0.920). An identical stop-gain *CFTR* mutation, c.1909C>T(p.Q637X), was identified in two bronchiectasis patients but none of the healthy subjects. *SCNN1A* and *SCNN1B* variants were identified in 6 bronchiectasis patients (3.1%) and 1 healthy subject (1.0%). The frequency of heterozygous mutations was comparable between healthy subjects and bronchiectasis patients (P=0.740) (*Table S6*).

Notably, four bronchiectasis patients harbored biallelic *CFTR* variants. Patient B168 harbored c.91C>T(p.R31C) and c.2684G>A(p.S895N) variants, patient B077 harbored IVS8 5T and c.1666A>G(p.I556V) variants, patient B179 harbored homozygous IVS8 5T variants, and patient B180

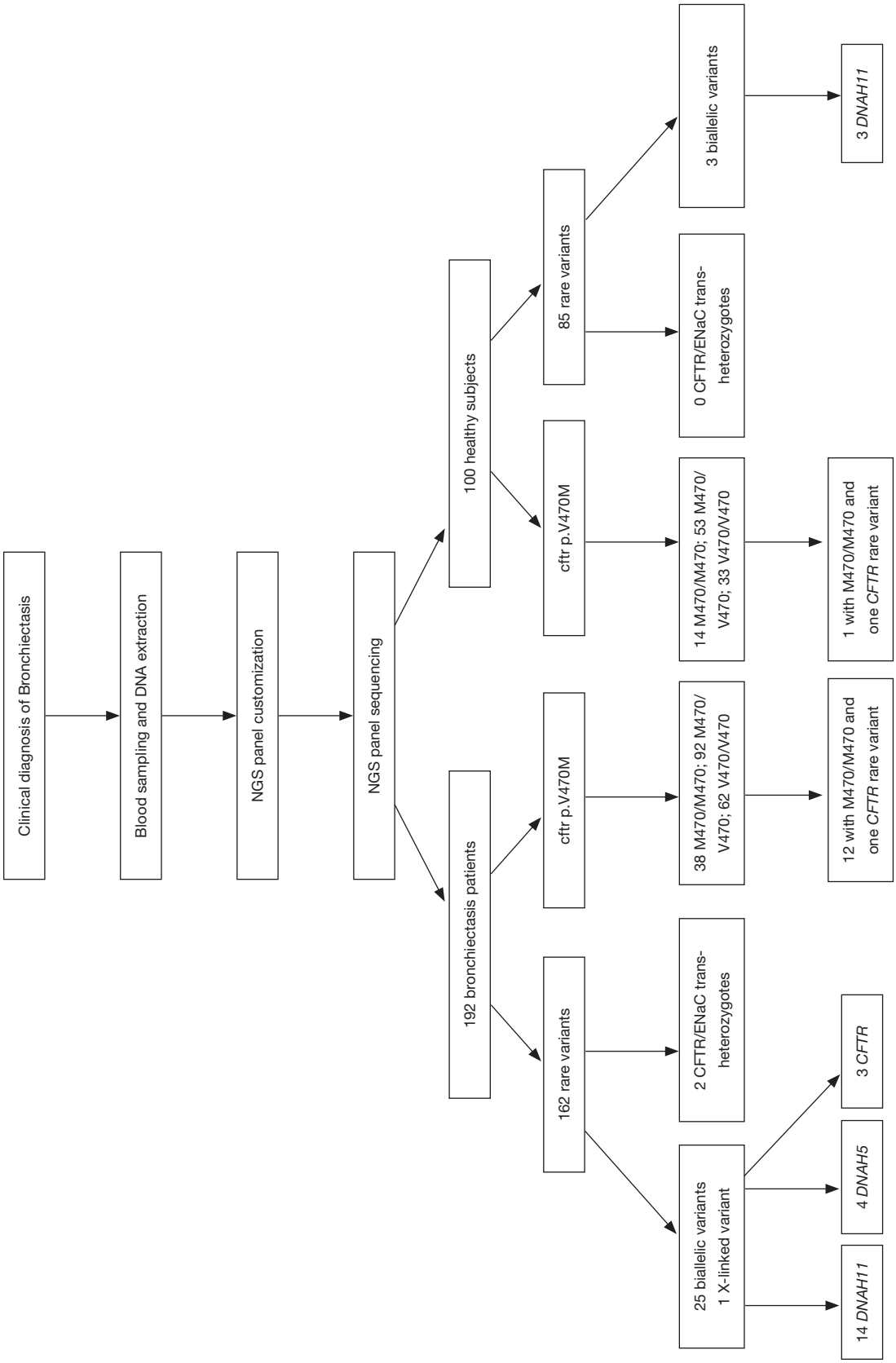

Figure 1 Strategies for screening potentially pathogenic mutations. Candidate mutations related to bronchiectasis were screened from literature and official websites of Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and Online Mendelian Inheritance in Man (http://omim.org/). Conservation and pathogenicity of mutations were subsequently determined.

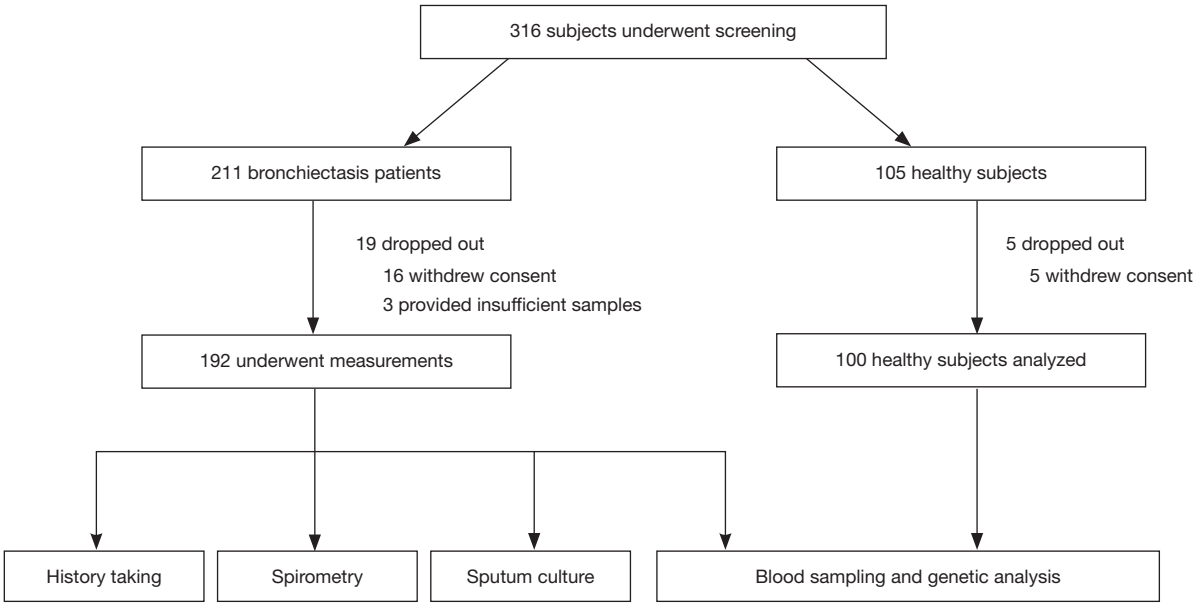

Figure 2 Study flow chart. Of 316 subjects who underwent screening, 19 bronchiectasis patients and 5 healthy subjects withdrew consent. Data from 192 bronchiectasis patients and 100 healthy subjects were analyzed.

harbored c.1666A>G(p.I556V) and c.3289C>T(p.R1097) variants (*Table 2*). However, none of the healthy subjects harbored biallelic *CFTR* variants.

Moreover, patient B142 harbored a c.T953G(p.I318S) missense mutation of *SCCN1A* and a c.A1666G(p.I556V) mutation of *CFTR*. Patient B180 harbored c.A1666G(p.I556V) and c.C3289T(p.R1097C) mutations of *CFTR*, and c.A806T(p.Y269F) of *SCCN1B*. None of the healthy subjects harbored *CFTR/ENaC* trans-heterozygote mutations.

p.V470M polymorphism

We identified 130 bronchiectasis patients (67.7%) who harbored p.M470 variants of *CFTR*. Homozygous p.M470 variants were identified in 38 bronchiectasis patients (29.2%), whereas heterozygous p.M470 variants in 92 bronchiectasis patients (70.7%). Sixty-seven healthy subjects (67.0%) harbored p.M470 variants. Of these, 14 subjects (20.8%) harbored homozygous p.M470 and 53 patients (79.1%) harbored heterozygous variants. No significant difference in the number of bronchiectasis patients with p.M470 carriers (P=0.902) or homozygous variants was found (P=0.209).

Of 92 bronchiectasis patients with heterozygous p.M470 variants, 20 (21.7%) harbored other rare *CFTR* variants. Of 38 bronchiectasis patients with homozygous p.M470 variants, 12 (31.5%) harbored miscellaneous *CFTR* mutations. Miscellaneous *CFTR* mutations were detected in 12 (22.6%) of 53 healthy subjects with heterozygous p.M470 variants and 1 (7%) of 14 healthy subjects with homozygous p.M470 variant.

Homozygous p.M470 and other *CFTR* variants were more frequently observed in bronchiectasis patients than in healthy subjects (6.3% *vs.* 1.0%, P=0.039) (*Table 3*).

PCD gene mutations

We detected 117 rare variants of PCD-associated genes in 101 bronchiectasis patients. When analyzing 20 PCD-associated genes, we identified 36 *DNAH11* variants (18 novel variants), 22 *DNAH5* variants (11 novel variants), nine *CCDC40* variants, seven *HEATR2* variants, six variants each of *DNAAF2* and *DRC1*, four variants each of *RSPH9* and *NME8*, three variants each of *CCDC114*, *CCDC39*, *DNAAF1*, *DNAAF3*, *LRRC6*, *RPGR* and *DNAI2*, two variants of *DNAI1*, and one *CCDC103* variant. Fifty-nine variants (27.3%) were identified for *DNAH11* and *DNAH5* (*Table S2*). Moreover, we found eight carriers of c.G1394A(p.R465Q) variant (rs201303241) for *HEATR2* in exon 6, which was absent in healthy subjects. Five healthy subjects harbored c.G1270A(p.A424T) variants in exon 6

Table 1 Basic characteristics of bronchiectasis patients

Parameter	Bronchiectasis patients (n=192)
Anthropometry	
Age (years)	45.2±14.1
Height (cm)	160.0 (12.0)
Weight (kg)	52.0 (12.4)
Body mass index (kg/m^2)	20.3 (4.3)
Females, n (%)	115 (59.9)
Never-smokers, n (%)	170 (88.5)
Disease characteristics	
Duration from onset of symptoms (years)	10.0 (15.0)
No. of bronchiectatic lobes	4.0 (3.0)
HRCT total score	7.0 (6.0)
Bronchiectasis severity index	5.0 (7.0)
FEV_1% predicted	69.1±23.9
Family history of any respiratory disease, n (%)	22 (11.5)
Family history of bronchiectasis, n (%)	11 (5.7)
Bronchiectasis etiology, n (%)	
Idiopathic	61 (31.8)
Post-infectious	53 (27.6)
Immunodeficiency	25 (13.0)
Asthma	8 (4.2)
Gastroesophageal reflux	6 (3.1)
Other known etiologies	16 (8.3)
Sputum bacteriology, n (%)	
Pseudomonas aeruginosa	63 (32.8)
Haemophilus parainfluenzae	15 (7.8)
Haemophilus influenzae	21 (10.9)
Other PPMs	20 (10.4)
Commensals	73 (38.0)

Numerical data are presented as mean ± standard deviation for normal distribution or otherwise median (interquartile range). Categorical data are expressed as number (percentage). None of the patients was using inhaled or oral antibiotics at the time of the study. PPMs, the abbreviation for potentially pathogenic microorganisms. In our study, other PPMs are consisted of *Staphylococcus aureus*, Klebsiella spp. and other clinically significant bacteria (e.g., *Stenotrophomonas maltophilia, Escherichia coli, Sphingomonas paucimobilis, Alcaligenes faecalis subsp faecalis, Pseudomonas pseudoalcaligenes and Serratia marcescens*). Other known etiologies included Kartagener syndrome, rheumatoid arthritis, lung maldevelopment, chronic obstructive pulmonary disease, allergic bronchopulmonary aspergillosis, Young's syndrome, non-*Tuberculous mycobacteria* disease (n=1, *Mycobacteria avium* and *Mycobacteria intracelluleae* complex was detected on multiple occasions), yellow nail syndrome, aspergilloma, diffuse panbronchiolitis, and eosinophilic bronchiolitis. Cystic fibrosis was not routinely screened because of the extremely low prevalence in China according to the previous literature reports. In this study, bronchiectasis in the two patients with Kartagener syndrome was secondary to primary ciliary dyskinesia. FEV_1, forced expiratory volume in one second.

Table 2 Patient with homozygous p.V470M variant and other CFTR mutations

Exon	Mutation 1	ACMG	No. of bronchiectasis patients	ExAC_Freq	dbSNP	Mutation 2
Exon 20	c.G3205A(p.G1069R)	VUS	4	0.0002	rs200321110	M470/M470
Exon 6	c.A650G(p.E217G)	P	2	0.0039	rs121909046	M470/M470
Exon 20	c.G3205A(p.G1069R)	VUS	4	0.0002	rs200321110	M470/M470
Exon 17	c.G2770A(p.D924N)	VUS	1	7.42E-05	rs201759207	M470/M470
Exon 10	IVS85T	P	8	0.0107	rs73715573	M470/M470
Exon 17	c.G2684A(p.S895N)	VUS	2	0.0003	rs201864483	M470/M470
Exon 11	c.G1558A(p.V520I)	P	1	0.0002	rs77646904	M470/M470
Exon 20	c.G3205A(p.G1069R)	VUS	4	0.0002	rs200321110	M470/M470
Exon 6	c.G601A(p.V201M)	P	2	0.0002	rs138338446	M470/M470
Exon 6	c.A650G(p.E217G)	P	2	0.0039	rs121909046	M470/M470
Exon 6	c.G601A(p.V201M)	P	2	0.0002	rs138338446	M470/M470
Exon 10	IVS85T	P	8	0.0107	rs73715573	M470/M470

AA, amino acid; ExAC-Freq, Exome Aggregation consortium-frequency; dbSNP, Single Nucleotide Polymorphism Database; CFTR, cystic fibrosis transmembrane conductance regulator; ACMG, American College of Medical Genetics and Genomics; VUS, variant of unknown significance; P, pathogenic; LP, likely pathogenic.

which were not identified among bronchiectasis patients.

Twenty bronchiectasis patients (17.1%) harbored biallelic variants, of whom 14 (70.0%) had biallelic *DNAH11* variants and four (16.0%) harbored biallelic *DNAH5* variants (*Table 3*). Patients B39 and B176 harbored homozygous variants of c.1394G>A(p.R465Q) for *HEATR2* and c.13373C>T(p.P4458L) for *DNAH11*, respectively. Patient B188 harbored c.518G>A(p.G173D) for *RPGR*. All these bronchiectasis patients had dual missense mutations, except for a single missense mutation plus splice mutation in patient B002, and non-sense mutation in patient B012.

Sixty-nine variants of PCD-associated genes were detected in 60 healthy subjects. When analyzing 15 PCD-associated genes, we identified 36 *DNAH11* variants, 16 *DNAH5* variants, 11 *LRRC6* variants, six variants each for *CCDC40*, *DRC1*, and *HEATR2*, five variants each for *DNAI2* and *RPGR*, four *DNAAF3* variants, three variants each for *DNAAF1*, *DNAAF2* and *NME8*, and two variants each for *CCDC114*, *CCDC39* and *RSPH9*. Of these, 52 variants (45.6%) were identified for *DNAH11* and *DNAH5*.

Of 60 healthy subjects with missense mutations, 3 (5%) harbored biallelic *DNAH11* variants (*Table 3*).

Clinical manifestations of patients with dual mutations

Twenty-five bronchiectasis patients (13.0%) and three healthy subjects (3.0%) harbored biallelic rare variants of an identical gene (χ^2=7.62, P=0.006). Of 25 bronchiectasis patients, 4 (16.0%) harbored biallelic *CFTR* variants, 4 (16.0%) harbored biallelic *DNAH5* variants and 14 (56.0%) harbored biallelic *DNAH11* variants (*Table 4*).

Of 14 patients with biallelic *DNAH11* variants, 3 (21.4%) had severe bronchiectasis. The most common etiology was idiopathic (57.1%), followed by post-tuberculous (21.4%). Mean HRCT score was 8.3. Mean forced expiratory volume in one second (FEV_1) was 77.3% predicted. Eleven (78.6%) patients had symptom onset for 20–50 years. Eight, six and two patients had cystic bronchiectasis, *Pseudomonas aeruginosa* isolated from sputum, and a family history of bronchiectasis, respectively.

Of four patients with biallelic *DNAH5* variants, 3 (75.0%) had severe bronchiectasis. The most common etiology was idiopathic (75.0%). The mean number of bronchiectatic lobes was 3.8 and HRCT score 8.0. Mean FEV_1 was 71.7% predicted. Three patients (75.0%) had symptom onset for 20–50 years. Three, one and two patients had cystic bronchiectasis, *Pseudomonas aeruginosa* isolated from sputum, and a family history of bronchiectasis, respectively.

Of four patients with biallelic *CFTR* variants, 1 (20.0%) had severe bronchiectasis, 3 (60.0%) had idiopathic bronchiectasis. Mean HRCT score was 6.2. Four patients had cystic bronchiectasis. *Pseudomonas aeruginosa* was

Table 3 Summary of carriers of dual mutations of CFTR and PCD

Mutation category	Patient No.	Gene	Mutations	ACMG	Consequences
Bronchiectasis patients with two mutations of CF genes or trans CFTR/ENaC mutations	B077	*CFTR*	IVS8 5T; c.1666A>G (p.I556V)	LP;VUS	Splice site mutation; Missense mutation
	B168	*CFTR*	c.91C>T (p.R31C); c.2684G>A (p.S895N)	VUS;VUS	Two heterozygous missense mutations
	B142	*CFTR/SCNN1A*	c.1666A>G (p.I556V)/c.953T>G（p.I318S）	VUS;VUS	Two heterozygous missense mutations
	B179	*CFTR*	IVS8 5T/ IVS8 5T	P;P	Homozygous splice site mutation
	B180	*CFTR/SCNN1B*	c.1666A>G (p.I556V); c.3289C>T (p.R1097)/ c.A806T(p.Y269F)	VUS;LP/VUS	Two heterozygous missense mutations
Bronchiectasis patients with two mutations of PCD genes	B002	*DNAH5*	c.11986C>T (p.R3996C); c.975+6C>T	VUS;VUS	Missense mutation; Splice site mutation
	B012	*DNAH11*	c.2419G>C (p.D807H); c.12258C>A (p.Y4086X)	VUS;LP	Missense mutation; Nonsense mutation
	B036	*DNAH11*	c.2542G>A (p.V848M); c.9260A>G (p.K3087R)	VUS;VUS	Two heterozygous missense mutations
	B039	*HEATR2*	c.1394G>A (p.R465Q); c.1394G>A (p.R465Q)	LP;LP	Homozygous missense mutation
	B073	*DNAH11*	c.7292G>T (p.S2431I); c.9017C>T (p.T3006M)	VUS;VUS	Two heterozygous missense mutations
	B082	*DNAH11*	c.2419G>C (p.D807H); c.2542G>A (p.V848M)	VUS;VUS	Two heterozygous missense mutations
	B091	*CCDC40*	c.1372G>T (p.A458S); c.1373G>T (p.A458V)	VUS;VUS	Two heterozygous missense mutations
	B099	*DNAH11*	c.4306C>T (p.R1436W); c.6118C>T (p.R2040C)	VUS;VUS	Two heterozygous missense mutations
	B128	*DNAH5*	c.6428A>T (p.E2143V); c.2047C>T (p.R683W)	VUS;VUS	Two heterozygous missense mutations
	B142	*DNAH11*	c.2912A>G (p.D971G); c.11396T>C (p.I3799T)	VUS;VUS	Two heterozygous missense mutations
	B150	*DNAH5*	c.13073G>A (p.R4358Q); c.11569A>G (p.R3857G)	VUS;VUS	Two heterozygous missense mutations
	B151	*DNAH5*	c.13073G>A (p.R4358Q); c.11569A>G (p.R3857G)	VUS;VUS	Two heterozygous missense mutations
	B156	*DNAH11*	c.2542G>A (p.V848M); c.12071C>T (p.S4024F)	VUS;VUS	Two heterozygous missense mutations
	B163	*DNAH11*	c.9260A>G (p.K3087R); c.13175C>T (p.T4392M)	VUS;VUS	Two heterozygous missense mutations
	B167	*DNAH11*	c.9260A>G (p.K3087R); c.11647C>T (p.L3883F)	VUS;VUS	Two heterozygous missense mutations
	B170	*DNAH11*	c.2542G>A (p.V848M); c.11624A>G (p.K3875R)	VUS;VUS	Two heterozygous missense mutations
	B176	*DNAH11*	c.13373C>T (p.P4458L); c.13373C>T (p.P4458L)	P;P	Homozygous missense mutation
	B178	*DNAH11*	c.6905A>C (p.H2302P); c.13112C>T (p.P4371L)	VUS;VUS	Two heterozygous missense mutations
	B185	*DNAH11*	c.4898C>A (p.S1633Y); c.9260A>G (p.K3087R)	VUS;VUS	Two heterozygous missense mutations
	B188	*RPGR**	c.518G>A (p.G173D)	LP	Missense mutation
	B212	*DNAH11*	c.1300T>C (p.F434L); c.6983C>T (p.P2328L)	VUS;VUS	Two heterozygous missense mutations
Healthy subjects with two mutations of PCD genes	C037	*DNAH11*	c.2419G>C (p.D807H); c.6724C>G (p.L2242V)	VUS;VUS	Two heterozygous missense mutations
	C075	*DNAH11*	c.5882A>T (p.Q1961L); c.6902A>G (p.H2301R)	VUS;VUS	Two heterozygous missense mutations
	C057	*DNAH11*	c.1409G>A (p.R470H); c.5144G>A (p.R1715H)	VUS;VUS	Two heterozygous missense mutations

*, *RPGR* is located on the X chromosome (hg19 chrX: 38,143,702-38,186,788). Because patient B188 is a male, only one mutation was displayed herein. CFTR, cystic fibrosis transmembrane conductance regulator; PCD, primary ciliary dyskinesia; DNAH, dynein axonemal heavy chain; RPGR, retinitis pigmentosa GTPase regulator; DNAAF, dynein axonemal assembly factor; CCDC, coiled-coil domain containing; HEATR2, heat repeat-containing protein; ACMG, American College of Medical Genetics and Genomics; VUS, variant of unknown significance; P, pathogenic; LP, likely pathogenic.

Table 4 Clinical characteristics of bronchiectasis patients with dual mutations associated with DNAH11, DNAH5, and CFTR

Gene mutation	Patient No.	Etiology	Age (years)	BSI	No. of bronchiectatic lobes	HRCT score	Age of symptom onset (yrs)	NMCC (s)	Cystic bronchiectasis	*Pseudomonas aeruginosa* isolated	FEV_1% predicted (%)	Family history of bronchiectasis
Dual *DNAH11* mutation	B012	GERD	45	9	5	11	22	NA	Yes	No	52.4	No
	B036	Post-tuberculous	50	5	6	14	47	>1800	Yes	No	87.2	No
	B073	Idiopathic	37	1	2	4	35	1040	No	No	77.8	No
	B082	Idiopathic	23	1	2	4	22	930	No	No	73.2	No
	B099	Post-tuberculous	63	2	3	6	53	776	No	Yes	96.2	No
	B142	Post-measles	41	2	5	9	1	625	Yes	No	62.8	No
	B156	Idiopathic	41	4	3	7	17	581	Yes	Yes	107.7	No
	B163	Idiopathic	67	5	5	7	57	563	No	No	99.0	No
	B167	IgG_1 deficiency	23	10	4	11	5	553	Yes	No	54.9	Yes
	B170	Post-tuberculous	73	5	1	2	63	548	No	No	75.2	No
	B176	Idiopathic	27	6	6	16	7	523	Yes	Yes	44.3	No
	B182	Idiopathic	40	0	1	3	38	503	Yes	Yes	86.0	No
	B185	Idiopathic	37	0	3	5	27	499	No	Yes	112.0	Yes
	B212	Idiopathic	39	9	6	17	29	448	Yes	Yes	53.0	No
Dual *DNAH5* mutation	B002	Idiopathic	32	3	3	7	17	NA	No	No	69.3	No
	B128	Idiopathic	69	12	6	12	55	677	Yes	Yes	53.0	No
	B150*	Idiopathic	61	11	2	5	59	596	Yes	No	96.9	Yes
	B151*	GERD	59	9	4	8	56	593	Yes	No	67.6	Yes
Dual *CFTR* mutation	B077	Idiopathic	57	2	3	3	54	995	No	No	103.9	No
	B142	Post-measles	41	2	5	9	40	625	Yes	No	62.8	No
	B168	Idiopathic	70	5	2	5	67	551	Yes	No	63.2	No
	B179	Idiopathic	63	3	2	6	43	511	Yes	Yes	66.9	No
	B180	Post-tuberculous	55	10	4	8	53	506	Yes	No	92.6	No

*, patients No. B150 and B151 were sisters. NMCC being equal to or greater than 1,800 seconds was deemed abnormal. BSI, Bronchiectasis Severity Index; GERD, gastroesophageal reflux disease; CFTR, cystic fibrosis transmembrane conductance regulator; DNAH, Dynein axonemal heavy chain; NMCC, nasal mucociliary clearance time (measured with saccharine test); NA, not applicable.

isolated in one case. No family history of bronchiectasis was documented.

Patient B180 who harbored biallelic *CFTR* variants and one *ENaC* variant had greater disease severity (BSI =10) than patient B140, the carrier of *CFTR* plus *ENaC* variant (BSI =2). As a carrier of *SCNN1B*, Patient B180 had more severe bronchiectasis compared with patient B077 (BSI =2) and B168 (BSI =5), carriers of biallelic *CFTR* variants without *ENaC* mutation.

Bronchiectasis etiology in patients with biallelic mutations

Of 192 bronchiectasis patients, 26 (13.4%) harbored biallelic mutations or an X-linked pathogenic mutation. The underlying causes of bronchiectasis were idiopathic, post-infectious, gastroesophageal reflux, immunodeficiency and asthma in 16, 6, 2, 1 and 1 each of bronchiectasis patients, respectively.

Discussion

Our study analyzed 32 genes associated with CF and PCD in bronchiectasis. Patients harboring biallelic rare mutations accounted for 13.4% of the cohort. Compared with healthy subjects, homozygous mutation of p.V470M or *ENaC* plus other *CFTR* mutations were more frequently observed in patients with bronchiectasis. Compared with bronchiectasis patients with biallelic *DNAH11* or *CFTR* mutations, those with biallelic *DNAH5* mutation seemed more likely to have greater bronchiectasis severity.

Altered mucus property arising from *CFTR* defects and/or *ENaC* over-expression has been linked to bronchiectasis. Fajac *et al.* (26) identified five amino-acid changes (p.Ser82Cys, p.Asn288Ser and p.Pro369Thr in ENaCβ; p.Gly183Ser and p.Glu197Lys in ENaCγ) in eight bronchiectasis patients with heterozygous *ENaC* mutations, and one amino-acid change (p.Ser82Cys) in one patient with *CFTR* mutation. In our study, none of the biallelic *CFTR* variants was identified in healthy subjects; three of six variants (50.0%) that were identified among bronchiectasis patients were novel *ENaC* variants (including *SCNN1A*, *SCNN1B* and *SCNN1G*), whereas *SCNN1B* variant was detected in healthy subjects only (*Table S4*). However, *SCNN1A* mutations alone might be insufficient to result in bronchiectasis, because only the p.W493R-*SCNN1A* and p.F61L-*SCNN1A* mutations predisposed to CF-like lung disease via hyperreactive sodium absorption (27). Gain-of-function (auto-dominant) mutation of *ENaC* predisposed to CF via increased sodium absorption, whereas loss-of-function (auto-recessive) mutation did not invariably result in airway diseases despite perturbed ion transportation (28,29). Nonetheless, whether *ENaC* mutations belonged to gain- or loss-of-function mutation awaits further investigation.

One of the major pulmonary manifestations of cystic fibrosis is bronchiectasis. The frequency of cystic fibrosis is approximately 1 in 3,500 newborn Caucasians. However, cystic fibrosis has rarely been reported in Chinese patients, and there has been sparse research evidence from China. To our knowledge, fewer than 30 patients have been reported in the Chinese population. In our study, we identified four patients with biallelic *CFTR* variant but none of the biallelic *CFTR* variant was identified in healthy subjects, suggesting that *CFTR* mutations may have played a role in the pathogenesis of bronchiectasis in China. It has been reported that M470 CFTR proteins have a 1.7-fold increased intrinsic chloride channel activity compared with V470 CFTR proteins (30). Additionally, 90% of bronchiectasis patients with mutations harbored the more functional M470 allele (31). In our study, 12 bronchiectasis patients simultaneously harbored M470/M470 and other *CFTR* variants, as did in one healthy subject. This indicated that homozygous M470 mutation plus other *CFTR* variants might more readily predispose to bronchiectasis, although further investigations for validation are merited.

ENaC and *CFTR* mutations reportedly conferred synergistic effects on the development of diffuse bronchiectasis (32). Patient B142 harbored missense mutation c.T953G(p.I318S) of *SCCN1A* and c.A1666G (p.I556V) of *CFTR*; patient B180 harbored c.A1666G(p. I556V) and c.C3289T(p.R1097C) mutation of *CFTR*, and c.A806T(p.Y269F) of *SCCN1B*. None of these variants was detected among healthy subjects. Moreover, patient B180 who harbored biallelic *CFTR* mutations plus *SCNN1A* variant had greater disease severity (BSI =10) than patient B140 (BSI =2) who had *CFTR* plus *SCNN1B* variant, and patient B077 (BSI =2) and B168 (BSI =5) who harbored dual *CFTR* variants but no *ENaC* variant.

Several variants of PCD-associated genes leading to abnormal ciliary motility have been previously reported. *DNAH5* mutation accounted for 27–38% of PCD patients with outer dynein arm defects (33). Despite a smaller proportion of PCD cases being affected and more subtle changes in ciliary ultrastructure, *DNAH11* mutation contributed to rigid and hypermotile cilia which hampered mucus clearance (18). Other mutations, including *RSPH9*

and *DNAI1*, have been implicated in the pathogenesis of bronchiectasis (17). Through sequencing of PCD-associated auto-recessive mutations (apart from *OFD1* and *RPGR* located in X-chromosomes), we identified 10.9% (n=21) of patients with at least one mutation (*DNAH11*: 7.3%, *DNAH5*: 2.6%). Of these, 14 cases harbored dual biallelic *DNAH11* heterozygous mutation and four harbored dual biallelic *DNAH5* heterozygous mutation (*Table 2*). Nonetheless, none of the bronchiectasis patients harbored *DNAI1* mutation. Intriguingly, patients with *DNAH5* mutation had greater disease severity compared with those with *DNAH11* mutation (mean BSI: 8.8 *vs.* 4.2), which corroborated with the findings that *DNAH5* mutation led to outer dynein arm defects and that *DNAH11* mutation may have limited negative impacts on ciliary ultrastructure (normal or near-normal ultrastructural changes) or motility. Moreover, we have identified eight bronchiectasis patients with *HEATR2* mutation (c.1394G>A (p.R465Q), heterogeneous mutation in seven cases), which was absent in healthy subjects, indicating that screening for *HEATR2* mutation should be recommended.

Bronchiectasis is a heterogeneous disease with limited therapeutic options (3). Despite exhaustive etiologic assessment, underlying causes cannot be determined in approximately 50% of cases (34). Identifying etiologies might lead to changes in patient's management (35). In our study, genetically susceptibility was revealed in 13.4% of patients with idiopathic bronchiectasis. Therefore, genetic testing complements conventional etiologic assessment by offering novel molecular information which would stimulate more in-depth research regarding host-defense defects in, and provide novel targets for future interventions of, bronchiectasis.

Nevertheless, we have included uniformly the Chinese Han population, which have limited the generalizability of our findings, as evidenced by the predominance of CFTR mutation in both healthy subjects and bronchiectasis patients. We might have underestimated some clinically significant mutations. Our findings cannot address whether other pathways (e.g., immunodeficiency, airway inflammation) are associated with bronchiectasis. Exploration of the association between genetic mutations of the immune system may help elucidate the mechanisms underlying how immunodeficiency contributes to bronchiectasis pathogenesis. The consequence of mutations has yet to be verified with sweat tests, nasal potential difference measurement and ciliary tests. The putative links between genetic mutations and bronchiectasis await verification with *in vivo* and *in vitro* models.

In conclusion, we have identified mutations associated with defective airway host-defense in bronchiectasis. Further development and functional validation of genetic testing for bronchiectasis is warranted.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: Ethics approval was obtained from Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University. Written informed consent was obtained.

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Methods

Summarized methods for determining bronchiectasis etiologies

Patients were meticulously inquired the past and present history, followed by physical examination, chest (and nasal, if indicated) HRCT assessment, spirometry, diffusing capacity measurement, venous blood sampling (blood routine test and immunoglobulins), sputum collection and culture, saccharine test and simplified reflux questionnaire interview. Patients susceptible of having gastroesophageal reflux disease (GERD), aspergillosis, asthma or chronic obstructive pulmonary disease (COPD) underwent 24-hour esophageal PH monitoring, serum total and *Aspergillus fumigatus*-specific IgE test, and bronchial dilation test, respectively. Hemagglutinin and auto-antibodies were tested for susceptible cases with diffuse panbronchiolitis and connective tissue diseases, respectively. Other diagnostic tests (e.g., bronchoscopy, gastroscopy) could be performed if appropriate (1,4,36).

Sputum bacteriology

Fresh sputum was sampled during hospital visits. Following removal of debris in oral cavity, patients expectorated into sterile container for bacterial culture. Hypertonic saline (3–5%) induction, previously validated, was applied as appropriate. Sputum was sent for bacterial culture within 2 hours of sampling.

Blood and chocolate agar plates (Biomeurix, France) were adopted as culture media. Fresh sputum was homogenized with SPUTASOL (Oxoid SR089A, Cambridge, UK) and serially diluted with natural saline at concentrations of 10^{-4}, 10^{-5} and 10^{-6}. This was followed by addition of 10μl respective diluent to the plates with micropipette tube and inoculation using 10 μL standardized rings. Plates were positioned in thermostatic box containing 5% carbon dioxide at 37 degrees for overnight incubation (20).

Assessment of chest HRCT scores

Chest HRCT at collimation of 2 mm or less within 12 months was captured. Bronchiectasis was diagnosed if the internal diameter of bronchi was greater than accompanying pulmonary artery. Miscellaneous signs of bronchiectasis included the lack of normal bronchial tapering along travel on sequential slices and (or) visible bronchi within 10 mm to the pleura. The HRCT score was assessed on a lobar basis, with lingular lobe being regarded as a separate lobe. For an individual lung lobe, the radiological severity of bronchiectasis was scaled by using modified Reiff score. The maximal total score was 18 for a total of six lobes (1,4,36).

Spirometry

Spirometry was conducted by using spirometers (QUARK PFT, COSMED Co. Ltd., Milan, Italy) according to American Thoracic Society/European Respiratory Society guidelines. Variation between the best two maneuvers was <5% or 200 mL in FVC and FEV_1, with maximal values being reported. Predicted values were derived from the model by Guan *et al.* (19,37).

Bronchiectasis severity index

Bronchiectasis severity index (BSI), a novel tool previously validated, was applied to determine the severity of bronchiectasis. The BSI was a composite of clinical parameters, including the age, body-mass index, prior exacerbation and prior hospitalization in the preceding year, modified Medical Research Council dyspnea score, FEV_1 predicted%, *Pseudomonas aeruginosa* infection, colonization with other potentially pathogenic microorganisms (PPMs, including *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Staphylococcus aureus*, *Klebsiella spp* and other clinically significant bacteria) and the number of bronchiectatic lobes. The BSI of ≤4, 5–8 and ≥9 denoted mild, moderate and severe bronchiectasis, respectively (22).

Strategies for screening mutants associated with bronchiectasis

Candidate mutants related to bronchiectasis pathogenesis were screened from literature reports (23-25) and official websites, including *Human Gene Mutation Data*base (http://www.hgmd.cf.ac.uk/ac/index.php) and *Online Mendelian Inheritance in Man* (http://omim.org/). The conservation and pathogenicity of mutants were subsequently determined (*Figure S1*).

First, we performed a pilot analysis on the sequencing data following gene sequencing with the Ion Torrent™ (Life Technologies Inc., NY, USA), which provided the automatic analyzing systems, the variantCaller™ and coverageAnalysis™ (Life Technologies Inc., NY, USA). The summary of mutation would be displayed through computerized automatic comparisons. We employed the wANNOVAR website for annotating the functional

consequences of gene mutants from the sequencing data. This has allowed researchers to screen for candidate mutants using the databases as delineated previously.

Next, the primary source file (*.bam) was generated and subsequently transformed into a readable Excel file (*.xls) following annotation, which was applied for the detailed comparison on candidate mutants with the original database to minimize errors (online: http://jtd.amegroups.com/public/system/jtd/supp-jtd.2018.04.134-1.pdf; http://jtd.amegroups.com/public/system/jtd/supp-jtd.2018.04.134-2.pdf). This would allow investigators to exclude the false-positive mutant loci with poor sequencing quality.

Finally, we targeted at screening for clinically significant mutations including missense, nonsense, frameshift mutation which were likely to lead to conformational or functional changes in proteins. Factors such as the potential pathogenicity (from previously published literature and databases such as NCBI and HGMD), the frequency of SNP (using the 1000 Genome database) and conversation (using SIFT and Polyphen2 software) should be taken into account when determining the panel of candidate mutant genes. In case no significant mutations were identified, we would then proceed to screen for candidate mutants according to their introns or UTR, as well as candidate mutants in the loci with synonymous mutation or splice mutation.

The panel of 32 candidate genes consisted of genes associated with bronchiectasis (*CFTR*, *SCNN1A*, *SCNN1B* and *SCNN1G*), PCD (*CCDC103*, *CCDC114*, *CCDC164*, *CCDC39*, *CCDC40*, *DNAAF1*, *DNAAF2*, *DNAAF3*, *DNAH11*, *DNAH5*, *DNAI1*, *DNAI2*, *DNAL1*, *HEATR2*, *LRRC6*, *OFD1*, *RPGR*, *RSPH4A*, *RSPH9*, and *TXNDC3*) and cystic fibrosis (CF) pathogenesis (*EFEMP2*, *ELN*, *FBLN5*, *FLCN*, *LTBP4*, *SERPINA1*, and *ATP7A*), and other pathogenic genes (*PAM* and *SLC34A2*) (*Tables S1-S3*).

Deoxyribonucleic acid extraction and mutation identification

Proband deoxyribonucleic acid (DNA), extracted from heparinized venous blood, was detected with NGS platform using customized Ion AmpliSeq™ Panel (Life Technologies Inc., NY, USA). This panel was designed to identify disease-causing mutations in exons and exon-intron boundaries (±50 bp) of bronchiectasis genes and those associated with PCD or cystic fibrosis pathogenesis.

First, fragment libraries were constructed according to manufacturer's instructions provided in the Ion Community for Ion Torrent™ sequencing using the panel and Ion AmpliSeq™ Library kit 2.0 (Life Technologies Inc., NY, USA).

Next, validation of enrichment and quantification of the resulting library were performed on the Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Sample emulsion polymerase chain reaction, emulsion breaking, and enrichment were performed on Ion OneTouch™ (Life Technologies Inc.) using Ion OneTouch™ 200 Template Kit (Life Technologies Inc., NY, USA). Ion Sphere™ Particles (ISPs) were recovered and template-positive ISPs were enriched using Dynabeads® MyOne™ Streptavidin C1 beads (Life Technologies Inc., NY, USA). ISP enrichment was confirmed using Qubit® 2.0 fluorometer (Life Technologies Inc., NY, USA), and the sample was prepared for sequencing using Ion PGM™ 200 Sequencing Kit protocol (Life Technologies Inc., NY, USA). The sample was then loaded on Ion 316™ chip and sequenced on Ion Torrent PGM™ (Life Technologies Inc., NY, USA). Softwares including Torrent Suite™ 4.0 (Life Technologies INc., NY, USA), SeattleSeq Annotation 9.00 (Seattle, WA, USA) and Integrative Genomics Viewer 2.1 (IGV, Broad Institute of MIT and Harvard, Cambridge, MA, USA) were utilized to perform bioinformatics analysis, including optimized signal processing, base calling, sequence alignment, and variant analysis.

Finally, pathogenic variants were confirmed by Sanger sequencing using ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Nomenclature for description of mutations was based on http://www.hg*vs*.org/mutnomen/, using NM_000138.4 as reference sequence.

Results

Mutants associated with CF

Eighteen CF mutants of 21 alleles were detected in 21 of bronchiectasis subjects. Of 100 healthy subjects, 9 CF mutants were detected in 11 cases (*Table S4*).

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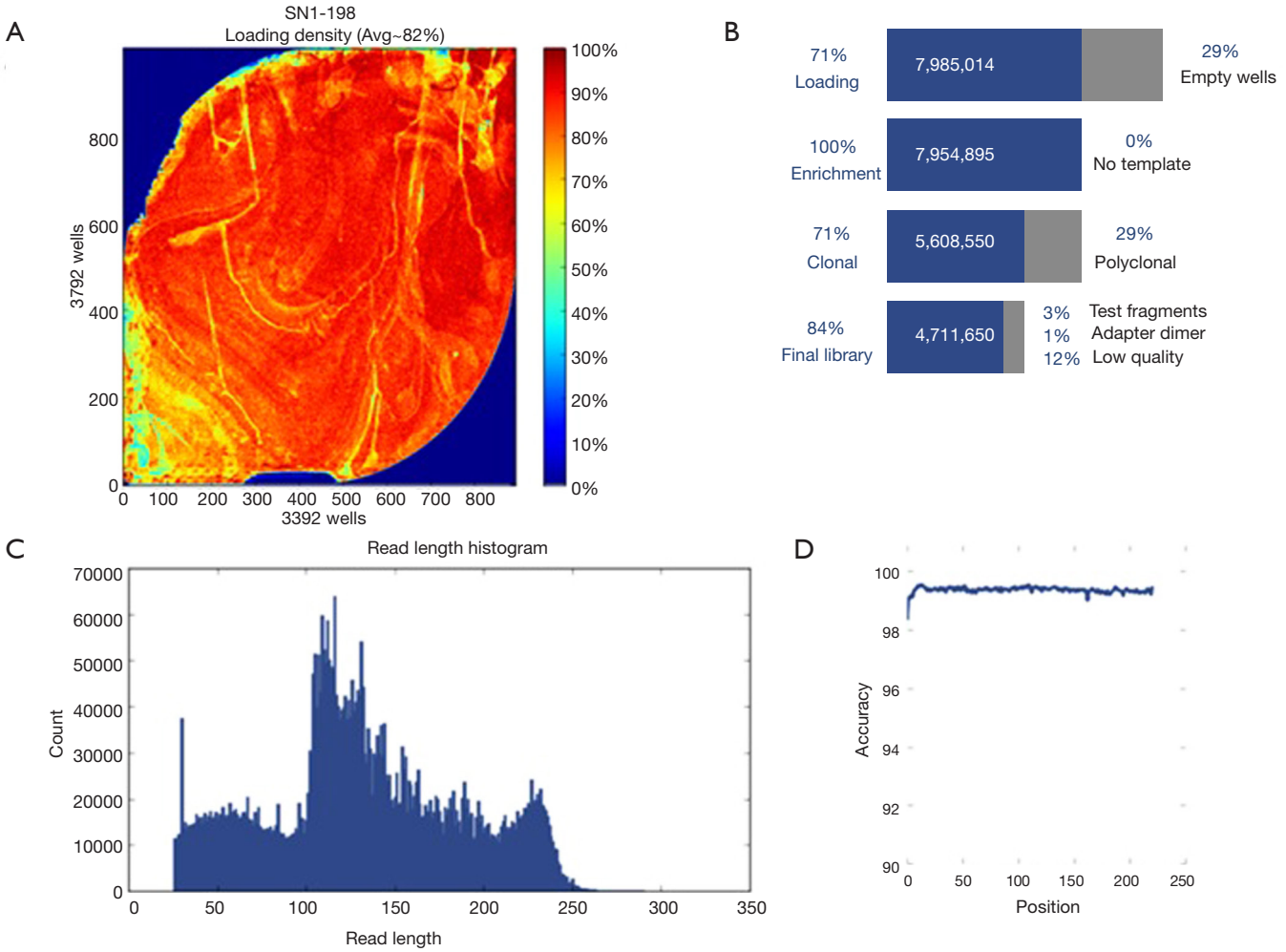

Figure S1 Bronchiectasis panel sequencing performance. (A) Ion sphere particles (ISPs) density diagram, showing the rate of ISPs for sequencing, with red portions indicating the positive ISPs (which could yield the sequencing data); (B) summary information of ISPs for sequencing; the percentage of active ISPs on individual dots for the gene chip was referred to as loading in the figure; (C) average read length for the ISPs for sequencing; (D) coverage of ISPs for sequencing.

Table S1 Common disease spectra associated with gene mutations

Genetic disorders	Disease	Type of disease	Genetic characteristics	Common clinical manifestation
EFEMP2, ELN, FBLN5, FBLN4, ATP7A	Congenital cutis laxa	Pulmonary cystic disorders	Autosomal dominant	Cutis laxa/pulmonary bulbs/emphysema/herniation/intestinal diverticulum
FLCN	Birt-Hogg-Dubé syndrome	Pulmonary cystic disorders	Autosomal dominant	Pulmonary bulbs/ renal malignancy/ cutaneous fibroma
LTBP4	NA	Pulmonary cystic disorders	NA	Respiratory distress/pulmonary cystic disorders or atelectasis/diaphragmatic herniation/tracheochondriomalacia
SERPINA1	α1-antitrypsin deficiency	Pulmonary cystic disorders	Autosomal recessive	Childhood pulmonary bulbs/liver fibrosis/necrotic subcutaneous steatitis
CFTR	Cystic fibrosis	Bronchiectasis	Autosomal recessive	Bronchiectasis/pancreatic insufficiency/congenital vas deference
CCDC39, CCDC40, DNAAF1, DNAAF2, DNAH11, DNAH5, DNAI1, DNAI2, DNAL1, RSPH4A, RSPH9	Primary ciliary dyskinesia	Bronchiectasis	Autosomal recessive	Rhinosinusitis/bronchiectasis/situs inversus
SCNN1A, SCNN1B, SCNN1G	Congenital sodium ion transporter anomaly	Bronchiectasis	Autosomal recessive	Clinical manifestations similar with cystic fibrosis
SLC34A2	Alveolar microlithiasis	Pulmonary interstitial diseases	SLC34A2-coding sodium-dependent phosphorylation co-transportation	Pulmonary multiple fine calcified nodules

CFTR, cystic fibrosis transmembrane conductance regulator; SCNN1A, sodium channel, epithelial, alpha subunit; SCNN1B, sodium channel, non-voltage-gated 1, beta subunit; SCNEG, sodium channel, epithelial, gamma subunit; DNAI1, dynein, axonemal, intermediate chain 1; DNAH5, dynein, axonemal, heavy chain 5; DNAH11, dynein, axonemal, heavy chain 11; DNAI2, dynein, axonemal, intermediate chain 2; DNAAF3, dynein, axonemal, assembly factor 3; NME8, thioredoxin domain-containing protein 3; DNAAF2, dynein, axonemal, assembly factor 2; RSPH4A, radial spoke head 4, chlamydomonas, homolog of, A; RSPH9, radial spoke head 9, chlamydomonas, homolog; DNAAF1, dynein, axonemal, assembly factor 1; CCDC39, coiled-coil domain-containing protein 39; CCDC40, coiled-coil domain-containing protein 40; DNAL1, dynein, axonemal, light chain 1; CCDC103, coiled-coil domain-containing protein 103; HEATR2, heat repeat-containing protein 2; LRRC6, leucine-rich repeat-containing protein 6; CCDC114, coiled-coil domain-containing protein 114; DRC1, dynein regulatory complex, subunit 1, chlamydomonas, homolog; ELN, elastin; FBLN5, fibulin 5; EFEMP2, EGF-containing fibulin-like extracellular matrix protein 2; LTBP4, latent transforming growth factor-beta-binding protein 4; OFD1, OFD1 GENE; RPGR, retinitis pigmentosa GTPase regulator; SLC34A2, solute carrier family 34 (sodium/phosphate cotransporter), member 2; FLCN, folliculin; SERPINA1, serpin peptidase inhibitor, clade a, member 1; ATP7A, ATPase, Cu^{2+}-transporting, alpha polypeptide. NA, not applicable.

Table S2 Gene mutations leading to primary ciliary dyskinesia

Gene	Chromosome location	Electron microscopic characteristics of biallelic gene mutation	Proportion of biallelic gene mutation	OMIM code#
DNAH5	5p15.2	ODA defect	Accounting for 15–21% of patients with PCD, and 27–38% of PCD patients with ODA defect	608644
DNAI1	9p21-p13	ODA defect	Accounting for 2–9% of patients with PCD, and 4–13% of PCD patients with ODA defect	244400
DNAI2	17q25	ODA defect	Accounting for 2% of patients with PCD, and 4% of PCD patients with ODA defect	612444
DNAL1	14q24.3	ODA defect	NA	614017
CCDC114	19q13.32	ODA defect	Accounting for 6% of PCD patients with ODA defect	615038
TXNDC3 (*NME8*)	7p14-p13	Partial ODA defect	NA	610852
DNAAF1 (*LRRC50*)	16q24.1	ODA + IDA defect	Accounting for 17% of PCD patients with ODA and defect	613193
DNAAF2 (*KTU*)	14q21.3	ODA + IDA defect	Accounting for 12% of PCD patients with ODA and defect	612517, 612518
DNAAF3 (*C19ORF51*)	19q13.42	ODA + IDA defect	NA	606763
CCDC103	17q21.31	ODA + IDA defect	NA	614679
HEATR2	7p22.3	ODA + IDA defect	NA	614864
LRRC6	8q24	ODA + IDA defect	Accounting for 11% of PCD patients with ODA and defect	614930
CCDC39	3q26.33	IDA defect and disoriented axonemal cilia	Accounting for 36–65% of cases with IDA defect and disoriented axonemal cilia in patients with PCD	613798
CCDC40	17q25.3	IDA defect and disoriented axonemal cilia	Accounting for 24–54% of cases with IDA defect and disoriented axonemal cilia in patients with PCD	613808
RSPH4A	6q22.1	Mostly normal, with central axonemal defect in a minority of cases	NA	612649
RSPH9	6p21.1	Mostly normal, with central axonemal defect in a minority of cases	NA	612648
HYDIN	16q22.2	Mostly normal, occasionally with central axonemal defect	NA	610812
DNAH11	7p21	Normal	Accounting for 6% of patients with PCD, and 22% of PCD patients with normal ciliary ultrastructure	603339
RPGR	Xp21.1	Mixed	PCD with X-linked retinitis	300170
OFD1	Xq22	NA	PCD with X-linked intelligence disorders	312610
CCDC164 (C2ORF39)	2p23.3	N-DRC defect; central axonemal defect in a minority of cases	NA	312610

Currently, there have been various genetic mutants associated with PCD pathogenesis, we herein have listed the most common mutants with known functions. CA, central axoneme; IDA, inner dynein arm; NA, not available; N-DRC, nexin-dynein regulatory complex; ODA, outer dynein arm; PCD, primary ciliary dyskinesia.

Table S3 A list of mutants, coding proteins, chromosome location and disease manifestations associated with gene mutation

Gene	Protein	Chromosome location	Disease manifestations
CFTR	Cystic fibrosis transmembrane conductance regulator	7q31.2	Cystic fibrosis; sweat chloride elevation without CF; bronchiectasis with or without elevated sweat chloride 1
SCNN1A	Sodium channel, epithelial, alpha subunit	12p13.31	Bronchiectasis with or without elevated sweat chloride 2
SCNN1B	Sodium channel, non-voltage-gated 1, beta subunit	16p12.2	Bronchiectasis with or without elevated sweat chloride 1
SCNEG	Sodium channel, epithelial, gamma subunit	16p12.2	Bronchiectasis with or without elevated sweat chloride 3
DNAI1	Dynein, axonemal, intermediate chain 1	9p13.3	Ciliary dyskinesia, primary, 1, with or without situs inversus
DNAH5	Dynein, axonemal, heavy chain 5	5p15.2	Ciliary dyskinesia, primary, 3, with or without situs inversus
DNAH11	Dynein, axonemal, heavy chain 11	7p15.3	Ciliary dyskinesia, primary, 7, with or without situs inversus
DNAI2	Dynein, axonemal, intermediate chain 2	17q25.1	Ciliary dyskinesia, primary, 9, with or without situs inversus
DNAAF3	Dynein, axonemal, assembly factor 3	19q13.42	Ciliary dyskinesia, primary, 2
NME8	Thioredoxin domain-containing protein 3	7p14.1	Ciliary dyskinesia, primary, 6
DNAAF2	Dynein, axonemal, assembly factor 2	14q21.3	Ciliary dyskinesia, primary, 10
RSPH4A	Radial spoke head 4, chlamydomonas, homolog of, a	6q22.1	Ciliary dyskinesia, primary, 11
RSPH9	Radial spoke head 9, chlamydomonas, homolog	6p21.1	Ciliary dyskinesia, primary, 12
DNAAF1	Dynein, axonemal, assembly factor 1	16q23.3-q24.1	Ciliary dyskinesia, primary, 13
CCDC39	Coiled-coil domain-containing protein 39	3q26.33	Ciliary dyskinesia, primary, 14
CCDC40	Coiled-coil domain-containing protein 40	17q25.3	Ciliary dyskinesia, primary, 15
DNAL1	Dynein, axonemal, light chain 1	14q24.3	Ciliary dyskinesia, primary, 16
CCDC103	Coiled-coil domain-containing protein 103	17q21.31	Ciliary dyskinesia, primary, 17
HEATR2	Heat repeat-containing protein 2	7p22.3	Ciliary dyskinesia, primary, 18
LRRC6	Leucine-rich repeat-containing protein 6	8q24.22	Ciliary dyskinesia, primary, 19
CCDC114	Coiled-coil domain-containing protein 114	19q13.33	Ciliary dyskinesia, primary, 20
DRC1	Dynein regulatory complex, subunit 1, chlamydomonas, homolog	2p23.3	Ciliary dyskinesia, primary, 21
ELN	Elastin	7q11.23	Cutis laxa, AD
FBLN5	Fibulin 5	14q32.12	Cutis laxa, autosomal dominant 2
EFEMP2	EGF-containing fibulin-like extracellular matrix protein 2	11q13.1	Cutis laxa, autosomal recessive, type IB
LTBP4	Latent transforming growth factor-beta-binding protein 4	19q13.2	Cutis laxa, autosomal recessive, type IC
OFD1	Ofd1 gene	Xp22.2	Joubert syndrome 10
RPGR	Retinitis pigmentosa GTPase regulator	Xp11.4	Macular degeneration, X-linked atrophic
SLC34A2	Solute carrier family 34 (sodium/phosphate cotransporter), member 2	4p15.2	Pulmonary alveolar microlithiasis
FLCN	Folliculin	17p11.2	Pneumothorax, primary spontaneous; Birt-Hogg-Dube syndrome
SERPINA1	Serpin peptidase inhibitor, clade a, member 1	14q32.13	Emphysema-cirrhosis, due to AAT deficiency; Pulmonary disease, chronic obstructive, susceptibility to
ATP7A	Atpase, Cu^{2+}-TRANSPORTING, ALPHA POLYPEPTIDE	Xq21.1	Menkes disease; occipital horn syndrome; Spinal muscular atrophy, distal, X-linked 3

CFTR, cystic fibrosis transmembrane conductance regulator; SCNN1A, sodium channel, epithelial, alpha subunit; SCNN1B, sodium channel, non-voltage-gated 1, beta subunit; SCNEG, sodium channel, epithelial, gamma subunit; DNAI1, dynein, axonemal, intermediate chain 1; DNAH5, dynein, axonemal, heavy chain 5; DNAH11, dynein, axonemal, heavy chain 11; DNAI2, dynein, axonemal, intermediate chain 2; DNAAF3, dynein, axonemal, assembly factor 3; NME8, thioredoxin domain-containing protein 3; DNAAF2, dynein, axonemal, assembly factor 2; RSPH4A, radial spoke head 4, chlamydomonas, homolog of, A; RSPH9, radial spoke head 9, chlamydomonas, homolog; DNAAF1, dynein, axonemal, assembly factor 1; CCDC39, coiled-coil domain-containing protein 39; CCDC40, coiled-coil domain-containing protein 40; DNAL1, dynein, axonemal, light chain 1; CCDC103, coiled-coil domain-containing protein 103; HEATR2, heat repeat-containing protein 2; LRRC6, leucine-rich repeat-containing protein 6; CCDC114, coiled-coil domain-containing protein 114; DRC1, dynein regulatory complex, subunit 1, chlamydomonas, homolog; ELN, elastin; FBLN5, fibulin 5; EFEMP2, EGF-containing fibulin-like extracellular matrix protein 2; LTBP4, latent transforming growth factor-beta-binding protein 4; OFD1, OFD1 gene; RPGR, retinitis pigmentosa GTPase regulator; SLC34A2, solute carrier family 34 (sodium/phosphate cotransporter), member 2; FLCN, folliculin; SERPINA1, serpin peptidase inhibitor, clade a, member 1; ATP7A, ATPase, Cu^{2+}-transporting, alpha polypeptide.

Table S4 The number of mutants identified through PGM sequencing

Mutation type	Bronchiectasis patients (n=192)	Healthy subjects (n=100)
Non-synonymous SNV	277	232
synonymous SNV	138	123
Stop-gain	6	2
Frameshift insertion	1	8
Frameshift deletion	4	2
Non-frameshift variation	0	6
Unknown	21	20
Total	447	393

SNV, single nucleotide variants.

Table S5 Pathogenic mutants identified among bronchiectasis patients and healthy subjects

Category of genetic mutations	Bronchiectasis patients (n=192)			Healthy subjects (n=100)		
	No. of pathogenic mutants	No. of alleles with pathogenic mutants	Recessive/compound heterozygous mutants	No. of pathogenic mutants	No. of alleles with pathogenic mutants	Recessive/compound heterozygous mutants
CFTR/ENaC	25	59	4	12	23	0
PCD	117	208	21	62	95	3
CF	18	20	0	10	10	0
Total	162	287	25	85	132	3

CFTR, cystic fibrosis transmembrane conductance regulator; PCD, primary ciliary dyskinesia; ENaC, epithelial sodium ion channel; CF, cystic fibrosis.

Table S6 Mutations of CFTR, SCNN1A, SCNN1B and SCNN1G among bronchiectasis patients and healthy subjects

Gene	Exon	Nucleotide mutation	Protein change	Variant classification	Bronchiectasis patients (n=192)	Healthy subjects (n=100)	ExAC_Freq	dbSNP
CFTR	Exon 12	c.1666A>G	p.I556V	Missense	18	9	0.0029	rs75789129
CFTR	Exon 20	c.3205G>A	p.G1069R	Missense	4	1	0.0002	rs200321110
CFTR	Exon 6	c.650A>G	p.E217G	Missense	2	1	0.0039	rs121909046
CFTR	Exon 25	c.4056G>C	p.Q1352H	Missense	5	3	0.001	rs113857788
CFTR	Exon 17	c.2770G>A	p.D924N	Missense	1	0	7.42E–05	rs201759207
CFTR	Exon 17	c.2684G>A	p.S895N	Missense	2	1	0.0003	rs201864483
CFTR	Exon 11	c.1558G>A	p.V520I	Missense	1	0	0.0002	rs77646904
CFTR	Exon 18	c.2936A>C	p.D979A	Missense	1	0	2.48E–05	rs397508462
CFTR	Exon 14	c.1909C>T	p.Q637X	Stop-gain	2	0	–	rs397508320
CFTR	Exon 6	c.601G>A	p.V201M	Missense	2	0	0.0002	rs138338446
CFTR	Exon 4	c.374T>C	p.I125T	Missense	1	1	0.0006	rs141723617
CFTR	Exon 27	c.4262T>A	p.V1421E	Missense	1	0	–	–
CFTR	Exon 2	c.91C>T	p.R31C	Missense	1	3	0.0017	rs1800073
CFTR	Exon 20	c.3289C>T	p.R1097C	Missense	1	1	0.0002	rs201591901
CFTR	Exon 22	c.3592G>A	p.V1198M	Missense	1	0	1.65E–05	rs576710089
CFTR	Exon 15	c.2540A>G	p.N847S	Missense	0	1	8.25E–06	rs562851847
CFTR	Exon 14	c.1811C>T	p.T604I	Missense	0	1	–	rs397508308
CFTR	Exon 15	c.2552G>A	p.R851Q	Missense	0	1	0.0001	rs397508395
CFTR	Exon 14	c.2042A>T	p.E681V	Missense	0	1	0.0001	rs201295415
CFTR	Exon 19	c.3004A>T	p.I1002F	Missense	0	1	8.28E–06	–
SCNN1A	Exon 5	c.1252C>A	p.P418T	Missense	1	0	8.83E–06	–
SCNN1A	Exon 1	c.205G>A	p.D69N	Missense	1	0	1.65E–05	–
SCNN1A	Exon 3	c.953T>G	p.I318S	Missense	1	0	–	–
SCNN1A	Exon 1	c.28C>T	p.R10W	Missense	1	0	0.0001	rs531838244
SCNN1B	Exon 5	c.863A>G	p.N288S	Missense	1	0	8.24E–06	rs137852712
SCNN1B	Exon 2	c.268A>G	p.M90V	Missense	1	0	0.0001	rs150466803
SCNN1B	Exon 5	c.857C>T	p.S286L	Missense	0	1	0.0002	rs142531781

CFTR, cystic fibrosis transmembrane conductance regulator; SCNN1A, sodium channel, epithelial, alpha subunit; SCNN1B, sodium channel, non-voltage-gated 1, beta subunit; NA, not applicable.