Whole Exome Sequencing Accuracy in the Diagnosis of Primary Ciliary Dyskinesia

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ONLINE DATA SUPPLEMENT

METHODS

The study was carried out in a tertiary referral university medical center. The methodology of the study was approved by the Institutional Review Board, and consent was obtained from patients or their legal guardians.

Patients

Individuals with a chronic sinopulmonary disease evaluated between 2012 and 2019 in whom cystic fibrosis and immune deficiency were ruled out (normal laboratory evaluation including; total blood count, immunoglobulin and immunoglobulin subclasses levels, serologic response to previously administered vaccines, leukocyte immunophenotyping, complement test and in selected cases tests as appropriate, i.e nitroblue tetrazolium test for suspected chronic granulomatous disease), were prospectively recruited for this study. Patients were included if they consented to perform WES and had a very high clinical suspicion for PCD: Term born with chronic sinopulmonary symptoms since early childhood and one or more of the following criteria; (1) unexplained bronchiectasis, (2) a condition associated with PCD (situs inversus totalis or any heterotaxic syndrome), (3) a history of otherwise unexplained neonatal respiratory

distress. Using these clinical criteria, we expected a pre-evaluation sensitivity of above 70% for a PCD diagnosis in our cohort[1, 2].

Data and sample collection

A chart review for missing information was conducted, including previously performed diagnostic testing for PCD. Patients were offered to complete diagnostic evaluation and repeat tests with equivocal results if previously performed. All subjects underwent detailed clinical assessment, nNO measurements and TEM from nasal brush biopsy.

The nNO was measured using a NO Analyzer CLD 88SP, Fa. (Eco Medics, Duernten, Switzerland). In cooperative patients, nNO sampling was performed with palate closure maneuver according to ATS/ERS guidelines [3]. In non-cooperative patients, mainly preschool children, nNO was measured during tidal breathing [4]. In all patients in whom the nNO measurement was low, at least two repeated nNO measurements were performed during separate clinical visits. The highest recorded nNO level was used in the final analysis. Due to the inclusion of nNO measurements in preschool individuals, measured during tidal breathing, we report nNO concentration (parts per billion (ppb)) in addition to nNO production (nl/min). For cooperative patients nNO production (nl/min) can be calculated by multiplying the nNO concentration (PPB) by the sampling flow rate (0.33 $L \cdot min^{-1}$for the CLD device) [5]. A nNO production values of 30 $nL \cdot min^{-1}$ (90 ppb for uncooperating subjects) and 77 $nL \cdot min^{-1}$ (233 ppb for uncooperating subjects) have been both recommended as cut-off values for evaluation of PCD, the former with increased specificity and latter with increased sensitivity, and were both assessed.

A nasal brush biopsy was performed under local anesthesia or during a clinically indicated bronchoscopy and was immediately fixed in glutaraldehyde for TEM analysis. TEM was analyzed by a pathologist experienced in the diagnosis of PCD. Ciliary ultrastructure was described as either “hallmark” abnormal, abnormal but suspected secondary ciliary defect[6], inadequate, inconclusive (no specific hallmark abnormality or a discordance between results from different time points), or normal. Repeat biopsies were offered to patients with suspected secondary defects and inadequate or inconclusive results. As cell culture was not available, suspected secondary defects and inadequate or inconclusive results are reported as such.

Whole Exome Analysis

Following DNA extraction from whole blood, exonic sequences were enriched with the SureSelect Human All Exon 50 Mb V5 Kit (Agilent Technologies, Santa Clara, California, USA). Sequences were generated on a HiSeq2500 (Illumina, San Diego, California, USA) as 125-bp paired-end runs. Read alignment and variant calling were performed with DNAnexus (Palo Alto, California, USA) with the human genome assembly hg19 (GRCh37) as reference. Data analysis was performed using an in-house bioinformatics pipeline.

Definitions

A positive diagnosis, in accordance with current ERS guidelines[7], was defined as 1) the presence of a typical clinical phenotype (as listed in the inclusion criteria) plus a recognized ciliary ultrastructural defect with or without abnormal low nNO levels, or 2) a

typical clinical phenotype and the presence of two pathogenic variants in a known gene causing PCD.

A negative diagnosis was reported in patients with typical clinical phenotype if 1) an alternative diagnosis was established or 2) all laboratory diagnostic studies were not consistent with PCD (high nNO levels, normal ciliary ultrastructure on TEM and no pathogenic variants in a recognized ciliary gene).

An unresolved diagnosis was reported in patients with a typical clinical phenotype if nNO levels were low and ciliary ultrastructure was normal or inconclusive and no pathogenic variants in a ciliary gene were found.

Statistical Analysis

Demographic, clinical, and laboratory variables were summarized by standard descriptive statistics as means and standard deviations for continuous variables and percentages for nominal variables. Proportional differences were assessed using the Chi-squared and Fisher's exact tests for nominal variables and T-test and the Mann-Whitney for continuous variables. Two-sided P values<0.05 were considered to be statistically significant. The diagnostic accuracy of each laboratory test was determined based on the final diagnosis. Patients with an unresolved diagnosis were not excluded but considered as a negative diagnosis for PCD.

Sensitivity, specificity, positive and negative predictive values and area under the receiver operating characteristic (ROC) curve were calculated for each diagnostic evaluation as well as a combined evaluation of low nNO levels and WES. All analyses

were performed using SPSS 25 (IBM Corp, NY, USA) and STATA 15.1 (Stata Corp TX, USA).

Table E1: Variants in ciliary genes identified by WES in PCD patients

GENE	POSITION	TRANSCRIPT	HGVSc	HGVSp	dbSNP	ZYGOSITY	EXPECTED SIGNIFICANCE	REF.
DNAH11	7:21847599	NM_001277115.2	c.10264G>A	p.Gly3422Arg	rs764509824	Homozygous	VOUS	
DNAH11	7:21940758	NM_001277115.2	c.13436_13437insCTGTG	p.Val4480CysfsTer8		Homozygous	expected pathogenic (LOF)	
DNAH11	7:21940756	NM_001277115.2	c.13457_13461dup	p.Tyr4488LeufsTer7		Homozygous	expected pathogenic (LOF)	
DNAH11	7: 21765427	NM_001277115.2	c.7267-2A>T			Homozygous	pathogenic (LOF)	
DNAH11	7:21932181	NM_001277115.2	c.12667G>T	p.Glu4223Ter		Homozygous	pathogenic (LOF)	
DNAI1	9:34517334	NM_012144.4	c.1871delC	p.Pro624LeufsTer66	rs1168493593	Homozygous	expected Pathogenic	
DNAI2	17:72287221	NM_023036.6	c.674delA	p.Asn225ThrfsTer17		Homozygous	expected pathogenic (LOF)	
DNAI2	17:72305484	NM_023036.6	c.1304G>A	p.Trp435Ter	rs752924362	Homozygous	Pathogenic	[8]
DNAI2	17:72281177	NM_023036.6	c.184-2A>T			Compound heterozygous	expected pathogenic (LOF)	
DNAI2	17: 72306303	NM_023036.6	c.1494+1G>A			Compound heterozygous	expected pathogenic (LOF)	
DNAAF3	19:55677272	NM_001256714.1	c.323T>C	p.Leu108Pro	rs387907151	Homozygous	pathogenic	[9]
DYX1C1	15: 55783336	NM_130810.4	c.384_390delCGCACTA	pTyr128del		Homozygous	expected pathogenic (LOF)	
HYDIN	16:71065734	NM_001270974.2	c.2616_2617insTGGCACTGAC	p.Leu873TrpfsTer3		Homozygous	expected pathogenic (LOF)	[10]
HYDIN	16:70989305	NM_001270974.2	c.6289C>T	p.Gln2097Ter	rs774501536	Homozygous	expected pathogenic	
LRRC6	8:133645203	NM_012472.6	c.436G>C	p.Asp146His	rs200321595	Homozygous	pathogenic	[11]
LRRC6	8:133673804	NM_012472.6	c.79_80delTC	p.Ser27ValfsTer13	rs769220870	Homozygous	expected pathogenic (LOF)	
LRRC50	16:84203779	NM_178452.6	c.1349_1350insC	pAsn230Asp	rs397515339	Homozygous	pathogenic	[12]

MCIDAS	5:54516210	NM_001190787.3	c.1142G>A	p.Arg381His	rs797045152	Homozygous	pathogenic	[13]
RSPH4A	6:116949263	NM_001010892.3	c.1393C>T	p.Arg465Ter	rs755782051		pathogenic	[14]
RSPH9	6:43638659	NM_001193341.1	c.801_803GAA	p.Lys268del	rs397515340	Homozygous	pathogenic	[15]

* dbSNP: The Single Nucleotide Polymorphism Database; HGVSc: Human Genome Variation Society nomenclature, coding; HGVSp: Human Genome Variation Society, protein change; LOF: loss-of-function; Ref: Reference; VOUS: variant of unknown significance.

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