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, Duernnten, 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·min⁻¹ for the CLD device) [5]. A nNO production values of 30 nL·min⁻¹ (90 ppb for uncooperating subjects) and 77 nL·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).
| GENE      | POSITION       | TRANSCRIPT       | HGVS<sub>c</sub> | HGVS<sub>p</sub> | dbSNP         | ZYGOSITY       | EXPECTED SIGNIFICANCE | REF. |
|-----------|----------------|------------------|------------------|------------------|---------------|------------------|------------------------|------|
| DNAH11    | 7:21847599     | NM_001277115.2   | c.10264G>A       | p.Gly3422Arg     | rs764509824   | Homozygous      | VOUS                   |      |
| DNAH11    | 7:21904758     | NM_001277115.2   | c.13436_13437insCTGTG | p.Val4480CysfsTer8 |              | Homozygous      | expected pathogenic (LOF) |      |
| DNAH11    | 7:21904756     | 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       |                  |              | Homozygous      | 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]  |
| DYXIC1    | 15:55783336    | NM_130810.4      | c.384_390delCGCACTA | p.Tyr128del    |              | Homozygous      | expected pathogenic (LOF) |      |
| HYDIN     | 16:71065734    | NM_001270974.2   | c.2616_2617insTGGCAC TGAC | 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  | p.Asn230Asp     | rs397515339   | Homozygous      | pathogenic             | [12] |
| Gene   | Chromosome | NCBI Reference | Variant Code | Mutation          | rsID       | Consequence | Classification |
|--------|------------|----------------|--------------|-------------------|------------|-------------|----------------|
| MCIDAS | 5:54516210 | NM_001190787.3 | c.1142G>A    | p.Arg381His       | rs797045152| Homozygous  | Pathogenic     |
| RSPH4A | 6:116949263| NM_001010892.3 | c.1393C>T    | p.Arg465Ter       | rs755782051| Pathogenic  |                |
| RSPH9  | 6:43638659 | NM_00119341.1  | c.801_803GAA | p.Lys268del       | rs397515340| Homozygous  | Pathogenic     |

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