



## ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

# The importance of genomic profiling for differential diagnosis of pediatric lung disease patients with suspected ciliopathies

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## SUMMARY

**Introduction/Objective** Dysfunction of the axonemal structure leads to ciliopathies. Sensory and motile ciliopathies have been associated with numerous pediatric diseases, including respiratory diseases. Primary ciliary dyskinesia (PCD) is ciliopathy linked to the dysfunction of motile cilia. Motile ciliary dysfunction in childhood leads to chronic rhinosinusitis, persistent cough, neonatal respiratory distress, bronchiectasis, and *situs inversus (SI)* have 50% of patients. These symptoms are common among pediatric lung diseases, which additionally makes it difficult to establish the accurate diagnosis. The aim of the study was to point out the significance of genomic profiling for patients with suspected ciliopathies and to design a strategy for genomic analysis relevant for differential diagnosis of lung disease patients with suspected ciliopathies.

**Methods** We conducted a bioinformatic analysis of data generated by New Generation Sequencing (NGS) approach of 21 patients with final or suspected diagnosis of PCD. It was analyzed 93 genes: 29 PCD genes, 45 genes related to individual symptoms of lung diseases, and 19 genes related to sensory ciliopathies.

**Results** The algorithm we have designed, enabled us to establish the clinical and genetic diagnosis for 17/21 (80.95%) patients, among which 11/21 (52.38%) were PCD patients. In 3/21 (14.28%) patients we detected monoallelic variants in PCD disease-causing genes. In 6/21 (28.57%) patients, variants in genes for other pulmonary diseases were detected, and for one patient, genetic background of disease remained unclear.

**Conclusion** An improved strategy for easier and faster establishment of final diagnosis of ciliopathies is mandatory and includes both, clinical and genetic confirmation of disease.

**Keywords:** pediatric lung disease; ciliopathies; PCD; NGS; genomic testing

## INTRODUCTION

Aberrant function of the axonemal structure has been related to the class of disorders collectively known as “ciliopathies” which includes primary ciliary dyskinesia (PCD), Alstrom syndrome, Bardet-Biedl syndrome, Joubert syndrome, nephronophthisis polycystic kidney disease, polycystic liver disease, Meckel-Gruber syndrome, hydrocephalus, Jeune syndrome, and laterality defects [1–4]. PCD was the first disease associated with the aberrant function of motile cilia.

Primary ciliary dyskinesia [PCD (MIM 244400)] is a very clinically and genetically diverse group of disorders with aberrant ciliary motility resulting in respiratory tract disease. Iannaccone et al. [5] found that the disease is inherited in autosomal recessive or X-linked manner. Lucas et al. [6] found that aberrant ciliary function in infancy leads to neonatal respiratory distress in term infants, wet cough, chronic rhinosinusitis, hearing impairment, bronchiectasis, and about 50% of patients have *situs inversus (SI)*. Noone et al. [7] proposed an explanation for the association between PCD

and SI, that the normal function of cilia plays a role in regular organ orientation, whereas orientation of the organs in PCD is a random event due to abnormal function of cilia in early embryonic development. Although the diagnosis of PCD is much easier when abnormal placement of the internal organs is present, SI also can mislead physicians to suppose that patient has PCD, which does not have to be correct since SI is common within ciliopathies, can be isolated (without ciliary dysfunction), or can be an unusual state of cystic fibrosis (CF) [7, 8]. In the recent study, Driscoll et al. [9] described that bronchiectasis was reported in 37% of patients with autosomal-dominant polycystic kidney disease as a consequence of sensory cilia dysfunction. Morini et al. [10] said that, similar to cystic fibrosis, in the early years of life, an infant with ciliopathy may be undernourished, which makes the diagnosis a challenge.

The aim of our study was to point out the significance of genomic profiling for differential diagnosis of patients with comprehensive or partial clinical presentation of PCD, using next-generation sequencing (NGS) approach. This approach allows us to analyze causative

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and candidate PCD genes, but also genes involved in other pediatric lung diseases with clinical presentation similar to PCD, and to establish the correct diagnosis. We also wanted to design a strategy for genomic testing relevant for differential diagnosis of pediatric lung disease patients with suspected ciliopathies.

## METHODS

Written informed consent was obtained from all participants and the study protocol was approved by the Ethics Committee of the Dr Vukan Čupić Mother and Child Health Care Institute of Serbia in Belgrade, Serbia, and has, therefore, been performed in accordance with the standards laid down in the 1964 Helsinki Declaration and its later amendments. All patients were physically examined and in all of them ciliopathy was detected using optical microscopy. The samples were referred to the Institute of Molecular Genetics and Genetic Engineering for genetic profiling of clinically suspected ciliopathy.

### Diagnostic work up

We conducted genetic analysis of 29 (21 previously reported) subjects from 18 unrelated families of Serbian descent. Subjects with two or more clinical symptoms of PCD, as well as their relatives were included in this study. PCD diagnosis was made based on the clinical symptoms such as: chronic sinusitis, neonatal respiratory distress, bronchiectasis, recurrent pneumonia, SI, cilia motility, infertility, and all patients were treated at the Mother and Child Health Care Institute. Nasal cells sampled by nasal brushing procedure were used to examine cilia motility using optical microscope. Absence of CF was verified by analysis of the presence for *Pseudomonas aeruginosa* and sweat chloride test. The control group for candidate disease-causing variants detected using NGS approach, consisted of in-house collection of 69 subjects from general population of Serbia sequenced by NGS methodology.

### Genomic analysis

Genomic DNA obtained from 29 subjects (21 patients, two siblings and six parents) was isolated from peripheral blood by QIAampDNA Mini Kit (Qiagen GmbH, Hilden, Germany). Qubit 3.0 fluorimeter (Invitrogen, Carlsbad, CA, USA) was used for measuring the quality and quantity of isolated DNA. Qubit dsDNA HS (Invitrogen) Assay Kit with range of 0.2–100 ng was used. Patients were analyzed by the NGS approach using Clinical-Exome Sequencing TruSight One Gene Panel (Illumina, San Diego, CA, USA). The relatives were analyzed only for detected gene variants in probands by direct Sanger sequencing.

### A gene selection strategy and variant filtering

To identify the mutational profile involved in the pathogenesis of PCD, we analyzed disease-associated genes

according to the Online Mendelian Inheritance in Man (OMIM). We also searched PubMed for studies describing clinical symptoms and genetic background of PCD patients. The first search implied the following terms: “primary ciliary dyskinesia,” “immotile cilia syndrome,” “ciliary motility disorders,” “Kartagener syndrome,” “abnormalities in the ciliary ultrastructure,” “abnormalities in the beating pattern,” “underlying genetic causes of the ciliary dysfunction,” “genes encoding ODA components,” “genes related to assembling of dynein arms,” “genes encoding radial spokes or CP component,” “genes encoding proteins of the N-DRC,” “genetic testing in PCD,” “novel clinical phenotypes for PCD,” “male infertility in PCD,” etc. Then, besides the genes described in the literature, we added genes that are coding for proteins that participate in protein-protein interactions with PCD disease-associated proteins in accordance to String Interaction Network (<https://string-db.org/>), and genes from the same gene family as PCD disease-causing genes. So, the first step of the analysis comprised 29 genes: (*CCDC8*, *CCDC39*, *CCDC40*, *CCDC50*, *CCDC88*, *CCDC103*, *DNAAF1* (*LRRC50*), *DNAAF2* (*KTU*), *DNAAF3*, *DNAI1*, *DNAI2*, *DNAL1*, *DNAH1*, *DNAH5*, *DNAH9*, *DNAH11*, *DYNC1H1*, *HEATR2*, *HYDIN*, *LRRC6*, *NME1*, *NME8* (*TXNDC3*), *OFD1*, *RPGR*, *RSPH4A*, *RSPH9*, *SPAG16*, *SPAG17*, and *TCTE1*).

To identify mutational profile of patients in which we didn't find variants in above listed genes, we analyzed genes related to individual symptoms of the disease. We searched on PubMed for the following terms: “bronchiectasis,” “bronchiectasis causes,” “chronic cough,” “mucus production,” “increased mucus secretion,” “CF bronchiectasis,” “bronchiectasis without CF” “respiratory distress in term neonates,” “surfactant metabolism,” “highly expressed lung proteins,” etc. We selected 45 genes for these symptoms that were present in TruSight One Gene Panel: *ABCA3*, *ABCA1*, *ABCB1*, *ABCC3*, *ABCA10*, *ABCB11*, *ABCC4*, *ABCA12*, *ABCB4*, *ABCC6*, *ABCA13*, *ABCB6*, *ABCC8*, *ABCA2*, *ABCB7*, *ABCC9*, *ABCC1*, *ABCD1*, *ABCA4*, *ABCC11*, *ABCD4*, *ABCA7*, *ABCC2*, *ABCG1*, *ABCG2*, *ABCG5*, *ABCG8*, *CFTR*, *MUC1*, *MUC13*, *MUC2*, *MUC3A*, *MUC4*, *MUC5B*, *MUC6*, *MUC7*, *SFTPA1*, *SFTPA2*, *SFTPB*, *SFTPC*, *SFTPD*, *SCNN1A*, *SCNN1B*, *SCNN1G*, and *SLC26A9*.

The last group of analyzed genes in our genomic analysis was related to sensory ciliopathies, present in TruSight One Gene Panel, 19 genes in total: (*BBS1*, *BBS2*, *BBS3*, *BBS4*, *BBS5*, *BBS6*, *BBS7*, *BBS8*, *BBS9*, *BBIP10*, *KIF3A*, *KIF3B*, *KAP*, *IFTA*, *IFTB*, *IFT43*, *IFT80*, *IFT122*, and *TTC21B*). The searching terms were as follows: “diseases associated with ciliary dysfunction”, “ciliopathies”, “sensory ciliopathies”.

Also, all patients were analyzed for presence of homozygous or compound heterozygous variants in *CFTR* gene to exclude the possibility of CF as final diagnosis.

Overall, we have performed bioinformatic analysis of 93 genes (Table 1).

**Table 1.** In-house designed list of genes related to pediatric lung diseases associated with ciliopathies; list of 93 genes identified on Illumina Clinical-Exome Sequencing TruSight One Gene Panel

|        |        |       |        |         |        |         |        |       |        |         |       |
|--------|--------|-------|--------|---------|--------|---------|--------|-------|--------|---------|-------|
| ABCA1  | ABCB1  | ABCC3 | ABCG2  | BBS5    | CCDC50 | DNAH5   | HYDIN  | KIF3B | MUC6   | SCNN1   | SFTPB |
| ABCA10 | ABCB11 | ABCC4 | ABCG5  | BBS6    | CCDC8  | DNAH9   | IFT122 | LRRC6 | MUC7   | SCNN1B  | SFTPC |
| ABCA12 | ABCB4  | ABCC6 | ABCG8  | BBS7    | CCDC88 | DNAH11  | IFT43  | MUC1  | NME1   | SCNN1G  | SFTPD |
| ABCA13 | ABCB6  | ABCC8 | BBIP10 | BBS8    | CFTR   | DNAI1   | IFT80  | MUC13 | NME8   | SLC26A9 | TCTE1 |
| ABCA2  | ABCB7  | ABCC9 | BBS1   | BBS9    | DNAAF1 | DNAI2   | IFTA   | MUC2  | OFD1   | SPAG16  | TTC21 |
| ABCA3  | ABCC1  | ABCD1 | BBS2   | CCDC103 | DNAAF2 | DNAL1   | IFTB   | MUC3A | RPGR   | SPAG17  |       |
| ABCA4  | ABCC11 | ABCD4 | BBS3   | CCDC39  | DNAAF3 | DYNC1H1 | KAP    | MUC4  | RSPH4A | SFTPA1  |       |
| ABCA7  | ABCC2  | ABCG1 | BBS4   | CCDC40  | DNAH1  | HEATR2  | KIF3A  | MUC5B | RSPH9  | SFTPA2  |       |

## Bioinformatic analysis

Illumina Clinical-Exome Sequencing TruSight One Gene Panel includes all known disease-associated genes described in the OMIM database until 2013, designed to cover all exons and flanking intronic regions of 4813 genes (~62,000 exons). Illumina MiSeq machine was used to generate comprehensive genetic libraries. The software for data analyzing gained using this approach was Illumina Variant Studio 2.0 and 3.0 (Illumina). After automatic annotation of all samples, we have accessed to variant filtering. Within this Illumina software there are numerous information about the analyzed variant, such as genomic coordinates, classifications, conservation scores, cDNA, CDS, and protein positions, transcripts and consequences, but also information about allele frequencies (1000 Genomes and ExaC) and functional impact of variants (SIFT and PolyPhen2). Only variants that had allele frequencies less than 5%, predicted as damaging, and absent in 69 control samples (TruSight One base), were further analyzed and considered as candidate variants. If the analyzed genetic variant had frequency > 5% in European population, but wasn't detected in our control samples, and was predicted as pathogenic, we took it into account. The databases used to additionally determine candidate variants: VarSome [which includes the databases: ClinVar, classification according to American College of Medical Genetics and Genomics (ACMG)], gnomAD genomes, gnomAD exomes, The Human Gene Mutation Database (HGMD), 1000 Genomes Project, dbSNP, Exome Aggregation Consortium (ExaC), and Ensembl. Tools used for pathogenicity scoring were: MutationTaster, Deleterious Annotation of Genetic Variants Using Neural Networks (DANN), and Functional Analysis through Hidden Markov Models (FATHMM-MKL). The effects on protein level were examined with *in silico* tools Provean, SIFT, and PolyPhen2.

## RESULTS

### Strategy for differential diagnosis of pediatric lung disease patients with suspected ciliopathies

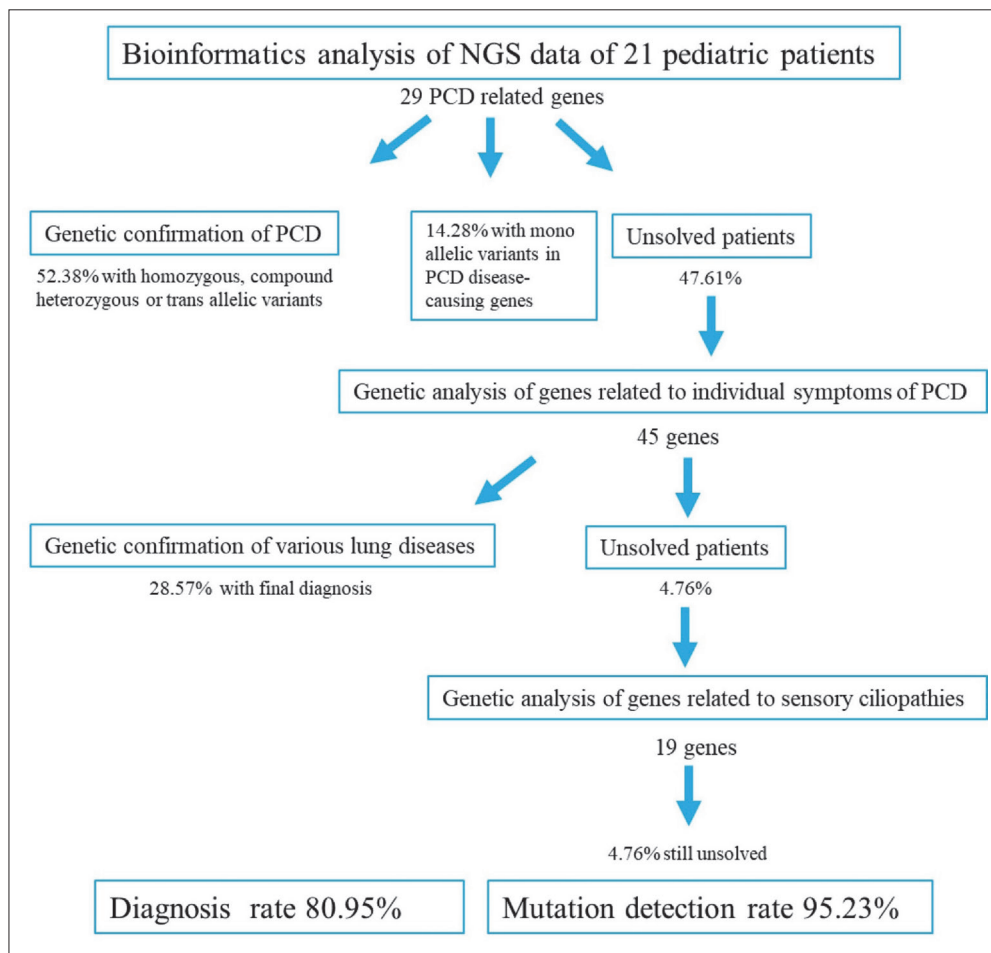
We have proposed an algorithm for differential diagnosis between PCD and other pediatric lung diseases with similar clinical presentation (Figure 1). Clinical diagnosis of PCD was the criterion for genomic profiling of the pa-

tients, and it was established when a patient exhibited two or more symptoms of the clinical presentation of PCD.

Using the NGS for analysis of 4813 genes, we generated large libraries with approximately nine thousand variants per patient. Overall, we have done bioinformatic analysis of 93 genes. First, we prioritized 29 PCD causative genes and candidate genes and established a diagnosis for 11/21 (52.38%) patients from nine unrelated families. Then we expanded our genomic analysis to 45 genes related to individual symptoms such as bronchiectasis, atopic asthma, sinusitis and neonatal respiratory distress, and established the diagnosis for 6/21 (28.57%) patients. Thus, total diagnostic rate in our study reached 80.95%. The last group of analyzed genes were 19 genes related to sensory ciliopathies, but we weren't able to detect homozygous, compound heterozygous or trans allelic pathogenic variants and establish the diagnosis in the remaining 4/21 (19.04%) patients. However, in 3/21 patients we have found mono allelic pathogenic variants within PCD disease-causing genes (14.28%). That resulted in only 1/21 (4.76%) patient, in whom the genetic background of the disease remained unidentified.

Andelković et al. [11] claimed that genes and variants detected in PCD related genes in 11/21 patients, and mono allelic variants detected in 3/21 patients were previously reported. Among six patients with variants in genes related to individual symptoms of the disease, three (50%) had the same mutation in *SCNN1A* gene (NM\_001159576.1, c. 1654T>C, p. Trp552Arg), in two out of three patients, this gene variant was associated with gene variant in *CFTR* gene (NM\_000492.3, c. 3485G>A, p.Arg1162Leu), while in the third patient it was present in homozygous state. In the remaining three patients, gene variants were detected in heterozygous state in *ABCA3* (NM\_001089.2, c.2125C>T, p.Arg709Trp), in homozygous state in *SLC26A9* (NM\_134325.2, c.514G>A, p.Val172Met), and in compound heterozygous state in *MUC2* (NM\_002457.2, c.5735G>T, p.Gly1918Val; c.8084T>G, p.Ile2683Ser) genes. All variants were characterized as pathogenic, damaging or deleterious according to various databases and *in silico* prediction scores and tools, and had allele frequency less than 5% in European population. Detected variants in genes related to bronchiectasis, CF, neonatal respiratory distress and asthma are listed in Table 2.

The algorithm we designed provided the mutation detection rate of more than 95%. It also contributed to the improvement of diagnosis rate from 52% (PCD patients) to 81% (all analyzed patients).



**Figure 1.** Proposed algorithm for differential diagnosis between PCD and other lung diseases with suspected ciliopathies;

Candidates for genetic testing were patients with situs inversus totalis or other situs abnormalities, with both upper and lower respiratory tract disease, history of unexplained neonatal respiratory distress, infertility in reproductive period, and immotility or abnormal cilia motility. Twenty-one patients had more than two above mentioned symptoms and they were analyzed using TruSightOne panel. We have conducted bioinformatics analysis of NGS data of all patients for presence of homozygous, compound heterozygous or trans allelic variants in 29 known PCD disease-causing and candidate genes. After we had established the diagnosis of PCD for 11/21 52.38% of patients, we extended our analysis on genes related to individual symptoms of the disease (45 genes), and genes related to sensory ciliopathies (19 genes). We reached the mutation detection rate of 95% (20/21), and diagnosis rate of 81% (17/21).

**Table 2.** Genetic variants detected in Serbian patients with pediatric lung diseases associated with ciliopathies (PCD patients not included); all identified genetic variants were numbered based on cDNA reference sequences and as recommended by the Human Genome Variation Society (<http://www.hgvs.org/mutnomen>)

| GENE    | dbSNP       | Genetic variants  |                   | Type     | VarSome allele frequency (%) <sup>a</sup> | ACMG score <sup>b</sup> | DANN <sup>b</sup> | SIFT <sup>c</sup> | Provean <sup>c</sup> | PolyPhen2 <sup>c</sup> | HGMD                   |
|---------|-------------|-------------------|-------------------|----------|---|-------------------------|-------------------|-------------------|----------------------|------------------------|------------------------|
|         |             | Nucleotide change | Amino acid change |          |   |                         |                   |                   |                      |                        |                        |
| ABCA3   | rs148671332 | c.2125C>T         | p.Arg709Trp       | missense | 0   | PP3                     | 0.9989            | Damaging          | Damaging             | Deleterious            | novel                  |
|         | rs41359254  | c.5735G>T         | p.Gly1918Val      | missense | 0.01                                      | US*                     | -                 | Damaging          | Damaging             | Deleterious            | novel                  |
| MUC2    | rs41386948  | c.8084T>G         | p.Ile2683Ser      | missense | 0.02                                      | US*                     | -                 | Damaging          | Damaging             | Deleterious            | novel                  |
| SLC26A9 | rs146704092 | c.514G>A          | p.Val172Met       | missense | 0   | PP3                     | -                 | Damaging          | Neutral              | Deleterious            | novel                  |
| SCNN1A  | rs5742912   | c.1654T>C         | p.Trp552Arg       | missense | 2.29                                      | PP3, BP6                | 0.9961            | Damaging          | Damaging             | Deleterious            | novel                  |
| CFTR    | rs1800120   | c.3485G>A         | p.Arg1162Leu      | missense | 0   | PP3, BP6                | 0.9985            | Damaging          | Damaging             | Deleterious            | ClinVar RCV000671007.1 |

<sup>a</sup>According to 1000 Genome Project and ExaC;

<sup>b</sup>ACMG and DANN scores: The detailed explanation of scores was given in the Supporting Information;

<sup>c</sup>SIFT, Provean, and PolyPhen2: *In silico* functional prediction tools



## DISCUSSION

In order to reach an exact diagnosis in patients that exhibited the clinical presentation of PCD, which is similar to clinical presentations of other pediatric pulmonary disorders associated with ciliopathy, we have established the strategy for differential diagnosis based on genomic profiling using NGS methodology. In this study, genomic analysis revealed that 52% of patients had PCD. Additionally, we established the diagnosis of other pediatric lung diseases for 29% of patients.

### Patients with lung diseases presenting overlapping clinical symptoms with PCD patients

In 6/21 patients without variants in PCD related genes, but with clinical presentation of PCD, extended genomic analysis revealed that this heterogeneous group have genetic background indicating other lung diseases, such as ectopic asthma, NRDS and bronchiectasis without CF. Pathogenic variants in homozygous, compound heterozygous, and trans allelic state were detected in *ABCA3*, *CFTR*, *MUC2*, *SCNN1A*, and *SLC26A9* genes.

Hanukoglu and Hanikoglu [12] reported that the *SCNN1A* gene encodes the alpha subunit of the ENaC (amiloride-sensitive epithelial sodium channel), a channel that allows the movement of sodium ions from the lumen into epithelial cells through the apical cell membrane. Fajac and Viel [13] reported in their previous study that mutations in *SCNN1* gene family might be deleterious for ENaC channel and lead to bronchiectasis, especially in patients that are trans-heterozygotes for ENaC/CFTR mutations. *SCNN1A* gene might be included on the list of potential gene candidates for PCD, since bronchiectasis is one of the well-known PCD clinical manifestations.

Our patient that harbored heterozygous genetic variant in *ABCA3* gene exhibited recurrent bronchiolitis (with hospitalization) and SI. Bronchiolitis affects the tiny airways, and as these airways become inflamed, they swell and fill with mucus, which make breathing difficult. Yamano et al. [14] stated that the *ABCA3* gene encodes a protein that is localized to the limiting membrane of lamellar bodies in alveolar type II cells. Matsumura et al. [15] indicated in their studies in surrogate cell model systems that *ABCA3* pathogenic gene variants impair the metabolism of surfactant by altering intracellular trafficking, the way of folding the *ABCA3* protein or impairing hydrolysis of ATP. Newborns with homozygous or compound heterozygous mutations in *ABCA3* gene develop progressive, lethal, neonatal-onset respiratory distress due to surface tension-lowering function of lung surfactant [16, 17]. Stahlman et al. [18] reported that in newborns with a single gene variant, if combined with developmental immaturity, it could reduce *ABCA3* gene expression below a functional threshold and the consequence is NRDS.

Patient with homozygous pathogenic variants in *MUC2* gene exhibits symptoms of asthma, bronchitis, and sinusitis. The protein encoded by *MUC2* gene is secreted and forms an insoluble barrier composed of mucous (<https://www.ncbi.nlm.nih.gov/gene/4583>). *MUC2* gene variants

may lead to an altered glycoprotein folding or variable glycosylation levels. Clinically, these changes may modify the function of mucin [19, 20, 21]. Kaliner et al. [22] reported that the mucus is overproduced in the respiratory tract during acute conditions of the disease, and in chronic conditions (bronchitis, asthma, CF, and sinusitis), thereby contributing to mucus blockade of the airways.

In one patient, we have found pathogenic genetic variant in *SLC26A9* gene. This genetic variant was not found in our in-house TruSight One base. *SLC26A9* functions as a Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchanger, a Cl<sup>-</sup> channel and a Na<sup>+</sup> coupled transporter [23, 24]. Chang et al. [23] reported that the *SLC26A9* protein is localized to epithelia of the lung and stomach. CFTR and *SLC26A9* have overlapping expression in lung epithelia and gut, making essential protein-protein interactions. Moreover, in a recent GWAS analysis of patients with CF, Sun et al. [25] pointed out that expression of human *SLC26A9* gene is associated with various CF phenotypes. Several studies have indicated that *SLC26A9* mutations may impart large (monogenic) or small (polygenic) phenotypic effects in stomach-related diseases, CF, and/or other disorders in humans. Thus, Chen et al. [26] concluded that the functional characterization of *SLC26A9* variants are clinically very important.

Despite a similar clinical presentation in PCD patients and patients affected with other lung diseases, when the diagnosis of PCD was excluded, and the correct diagnosis of bronchiectasis, NRDS and asthma was established, more adequate therapy was used for treatment of the diseases, and the quality of life for those pediatric patients was improved.

In the remaining three patients, monoallelic pathogenic variants were detected in above listed 93 genes. For one patient, we could not associate clinical presentation with alterations in any of the genes included in our algorithm. This suggests that our in-house made list of 93 genes should be increased. New studies and new knowledge will enable the establishment of the genetic background in those patients as well. It is also necessary to emphasize that large chromosomal rearrangements encompassing these 93 genes that could be present in our patients, cannot be detected using NGS methodology.

## CONCLUSION

Our study indicates the significance of genomic profiling for differential diagnosis between patients with comprehensive or partial clinical presentation of PCD and patients with other pediatric lung diseases. The strategy we designed includes NGS analysis of 93 genes relevant for symptoms present in ciliopathies. The proposed diagnostic algorithm contributed to the improvement of diagnosis rate from 52% (PCD patients) to 81% (all analyzed patients). Given the heterogeneity of possible symptoms associated with PCD and ciliopathies in general, there is no uniform approach to establish a precise diagnose. Modern high-throughput genetic approaches allow the causative biallelic mutations identification in about 60% of patients.

Although not yet applied for routine diagnostics, NGS turned out to be more efficient and cost-effective in diagnosing PCD and other pediatric lung diseases associated with ciliopathies, compared to traditional sequencing of single genes. An improved strategy for easier and faster establishment of final diagnosis of ciliopathies is mandatory and includes both, clinical and genetic confirmation of the disease. Our study is in accordance with this standpoint.

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## Значај геномског профилисања за диференцијалну дијагнозу педијатријских болесника са болестима плућа суспектних на цилиопатије

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### САЖЕТАК

**Увод/Циљ** Измењена функција аксонемалне структуре доводи до цилиопатија (моторних и сензорних), које су до сада повезане са бројним педијатријским поремећајима, укључујући и респираторне. Примарна цилијарна дискинезија (ПЦД) најчешћа је цилиопатија, која настаје као последица поремећаја у моторним цилијама. Промењена структура и/или функција моторних цилија доводи до неонаталног респираторног дистреса, хроничног влажног кашља, симптома назалне секреције, бронхоектазија, хроничне упале синуса и уха, а 50% болесника има и *situs inversus*. Ови симптоми су прилично уобичајени код мале деце и у другим стањима; стога је успостављање прецизне дијагнозе отежано. Циљ овог истраживања је указивање на значај геномског профилисања болесника и дизајнирање стратегије за генетичку анализу података код болесника суспектних на цилиопатије са клиничком сликом сличном другим болестима плућа.

**Метод** Спровели смо биоинформатичку анализу података добијених методом секвенцирања нове генерације 21 бо-

лесника са потврђеном или суспектном дијагнозом ПЦД-а. Анализирано је 93 гена: 29 ПЦД гена, 45 гена асоцираних са појединачним симптомима плућних болести и 19 гена асоцираних са сензорним цилиопатијама.

**Резултати** Дизајнирани алгоритам за генетичку анализу нам је омогућио да потврдимо клиничку и успоставимо генетичку дијагнозу код 17/21 (80,95%) болесника, међу којима је 11/21 (52,38%) ПЦД болесника. Код 3/21 (14,28%) болесника детектоване су моноалелске варијанте у ПЦД генима, код 6/21 (28,57%) болесника детектоване су варијанте у генима релевантним за друга плућна обољења, док је код 1/21 (4,76%) болесника генетичка основна болест остала неразјашњена.

**Закључак** Дизајнирање стратегије за лакше и брже успостављање коначне дијагнозе цилиопатија је обавезно и укључује и клиничку и генетичку потврду болести.

**Кључне речи:** педијатријске плућне болести; цилиопатије; ПЦД; секвенцирање нове генерације; геномско тестирање