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RESEARCH ARTICLE

A novel *DNAH5* variant in a Tunisian patient with primary ciliary dyskinesia

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Abstract. Primary ciliary dyskinesia (PCD) is a genetically heterogeneous hereditary disease caused by the structural abnormalities and dysfunction of motile cilia. The *DNAH5* is the most frequently mutated gene in PCD patients and hot spot exons were reported in this gene. Here, we aim to screen mutations in a set of five hot spot exons of *DNAH5* gene in a cohort of 10 clinically diagnosed Tunisian PCD patients using an optimized polymerase chain reaction-single-strand conformational polymorphism screening technique. Only one patient harboured a novel heterozygous variant in exon 63 (c.10767A>G), which was inherited from his father. This variant activates a cryptic splicing site. No deleterious mutation has been identified while screening the exons of the remaining patients. Our results show that the reported hot spot exons of *DNAH5* gene are not mutated in Tunisian PCD patients. This is probably due to the differences of ethnical background of the previously reported patients. Further investigations should be performed to identify the mutations underlying PCD in this group of patients.

Keywords. primary ciliary dyskinesia; *DNAH5* gene; hot spot; splicing; single-strand conformation polymorphism; screening.

Introduction

Primary ciliary dyskinesia (PCD, MIM: 244400) is an autosomal recessive, highly heterogeneous genetic disorder caused by the alteration of motile cilia mobility leading to defected muccociliary clearance and resulting in recurrent infections of the respiratory tract. This ciliopathy is characterized by the chronic upper and lower respiratory tract disease, bronchiectasis, sinusitis and otitis media. About 50% of the patients show an organ laterality defect (*situs inversus totalis* or *situs ambiguus*/*heterotaxy*) (Fassad *et al*. 2018; Benjamin *et al*. 2019). The diagnosis of this disease is often delayed mainly because the respiratory symptoms are common to other pediatric pathologies, such as cystic fibrosis in addition to the lack of a simple and cost-effective diagnostic test (Lucas *et al.* 2016; Shapiro *et al.* 2018).

More than 40 different genes have been identified in association with PCD (Bustamante-Marin *et al.* 2019). Theoretically, this number is able to grow since the cilium is composed of over 200 different proteins (Geremek *et al.* 2011; O'Toole *et al.* 2012).

Among the identified genes, dynein heavy chain 5 (*DNAH5*) is the most frequently mutated gene and is composed of 79 exons and encodes a protein of 4624 amino acids. The encoded protein is a component of the outer dynein arm (ODA); a ciliary structure involved in the generation of cilia motility (Hornef *et al.* 2006). Previous studies have reported mutations in five hot spot exons (34, 50, 63, 76 and 77) in *DNAH5* gene (Hornef *et al.* 2006; Djakow *et al*. 2012). Due to its large size, we aim to screen the five hot spot exons of *DNAH5* gene in a group of clinically diagnosed Tunisian PCD patients using an optimized polymerase chain reaction-single-strand conformational polymorphism (PCR-SSCP) technique.

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Subjects and methods

Subjects and study design

Here we performed a cohort study consisting of a group of 10 Tunisian clinically diagnosed PCD patients (belonging to seven independent families) and one healthy subject. All patients have been recruited between January to December 2014 from the pediatric department of Hedi-Chaker University Hospita, Tunisia. Written informed consents were obtained from the parents or the legal guardian of each patient.

The five hot spot exons of *DNAH5* (exons 34, 50, 63, 76 and 77) were selected for mutation screening from each patient by PCR-SSCP technique. Eventual identification of mutation was performed by Sanger sequencing.

DNA extraction

Genomic DNAs were isolated from 5 mL peripheral blood samples using the salting-out method (Miller *et al.* 1988) modified in our laboratory. The quantity and quality of the extracted DNAs were evaluated by Nanodrop ND-1000 measurements and agarose gel electrophoresis.

PCR

Amplifications were performed at an annealing temperature of 60°C in a 50 μL reaction containing 50 ng of the genomic DNA and 0.1 μM of each primer. Primers were designed to encompass the *DNAH5* hot spot exons (exons 34, 50, 63, 76 and 77). Primers sequences will be provided upon request.

PCR-SSCP analysis

As previously reported (Gasser *et al.* 2007; Mabrouk *et al.* 2018), a volume of 3 μL of PCR products ($\approx$50 ng) was diluted 3.5 fold in 40% formamide, 1x gel loading buffer and 8 μM forward and/or reverse PCR primers. The mixture is heated for 15 min at 95°C on a thermocycler heat block and then immediately snap-cooled into ice for 2 min. The samples were rapidly loaded on to an 8% nondenaturing polyacrylamide (29:1) gel supplemented with 5% glycerol. Electrophoresis was performed at 80 V for 18 h at 4°C in 0.5× TBE buffer. Gels were stained using SYBR Gold nucleic acid gel stain (Invitrogen MP 11494) following the manufacturer instructions.

Sanger sequencing

Sanger sequencing was performed to verify the absence of polymorphisms in the control DNA and to characterize the variants detected through SSCP screening in patients. Purified PCR products were directly sequenced using the PCR forward or both PCR primers in ABI 3730xl DNA Analyser. Sequence alignments were carried out using the NCBI-BLAST algorithm.

Results

Patients

Ten patients belonging to seven independent families were recruited in this study and consanguinity was reported in five families. At the time of the study, four patients among the 10 were in their childhood, 1–18 years old; and six were in their early childhood, <5 years. The sex ratio of the group was equal to 1 (5:5, male:female). All the 10 recruited patients have shown neonatal respiratory distress and eight had bronchiectasis. Laterality defect (*situs inversus*) was observed in seven patients. Sinusitis and otitis were reported in six and five patients, respectively. Differential diagnosis of cystic fibrosis was eliminated in all patients (see table 1).

DNAH5 hot spot exons screening

We carried out the screening of the five previously reported hot spot exons (34, 50, 63, 76 and 77) of *DNAH5* gene (Hornef *et al.* 2006) in 10 recruited patients. The PCR-SSCP

Table 1. Clinical features of the studied group of PCD patients.

Family	Patient	Sex	Consanguinity	Situs inversus	Airway disease
A	A1	F	Yes	Yes	NRD, BD, sinusitis
B	B1	M	No	Yes	NRD, BD, sinusitis, otitis
B	B2	F	No	Yes	NRD, BD, sinusitis, otitis
C	C1	F	Yes	Yes	NRD, sinusitis, otites
D	D1	F	Yes	Yes	NRD, BD, otitis
E	E1	M	No	No	NRD
F	F1	M	Yes	No	NRD, BD, sinusitis
F	F2	M	Yes	Yes	NRD, BD, otitis
G	G1	F	Yes	No	NRD, BD, sinusitis
G	G2	M	Yes	Yes	NRD, BD

F, female; M, male; NRD, neonatal respiratory distress; BD, bronchial dilatation.

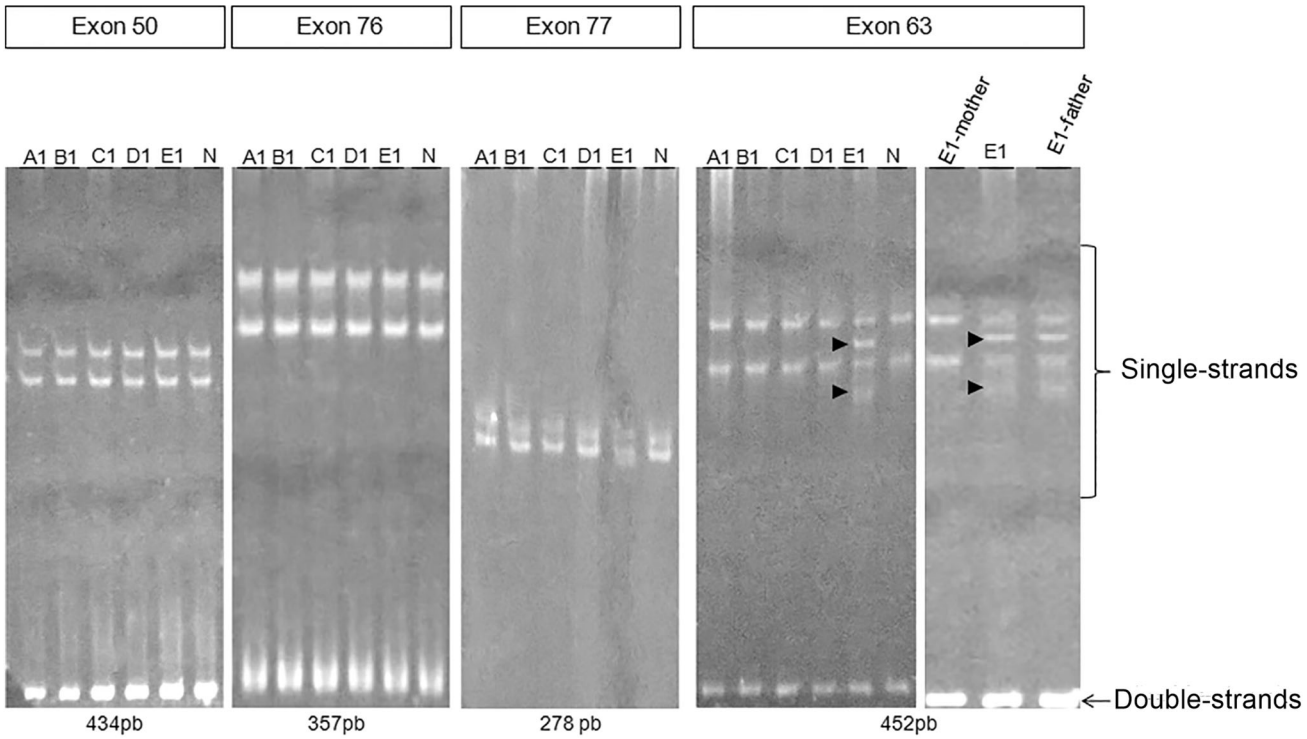

Figure 1. Results of PCR-SSCP screening of *DNAH5* exons 50, 63, 76 and 77. Patients are in lanes A1, B1, C1, and D1 and E1; N, healthy subject. All patients are homozygotes (wild-types) for all exons except patient E1 for exon 63 which is heterozygote indicating the presence of a variation. The heterozygous variant (indicated by the arrows) of patients E1 was detected in his father. Exons are indicated on the top and sizes (in bp) of amplicons are shown at the botom.

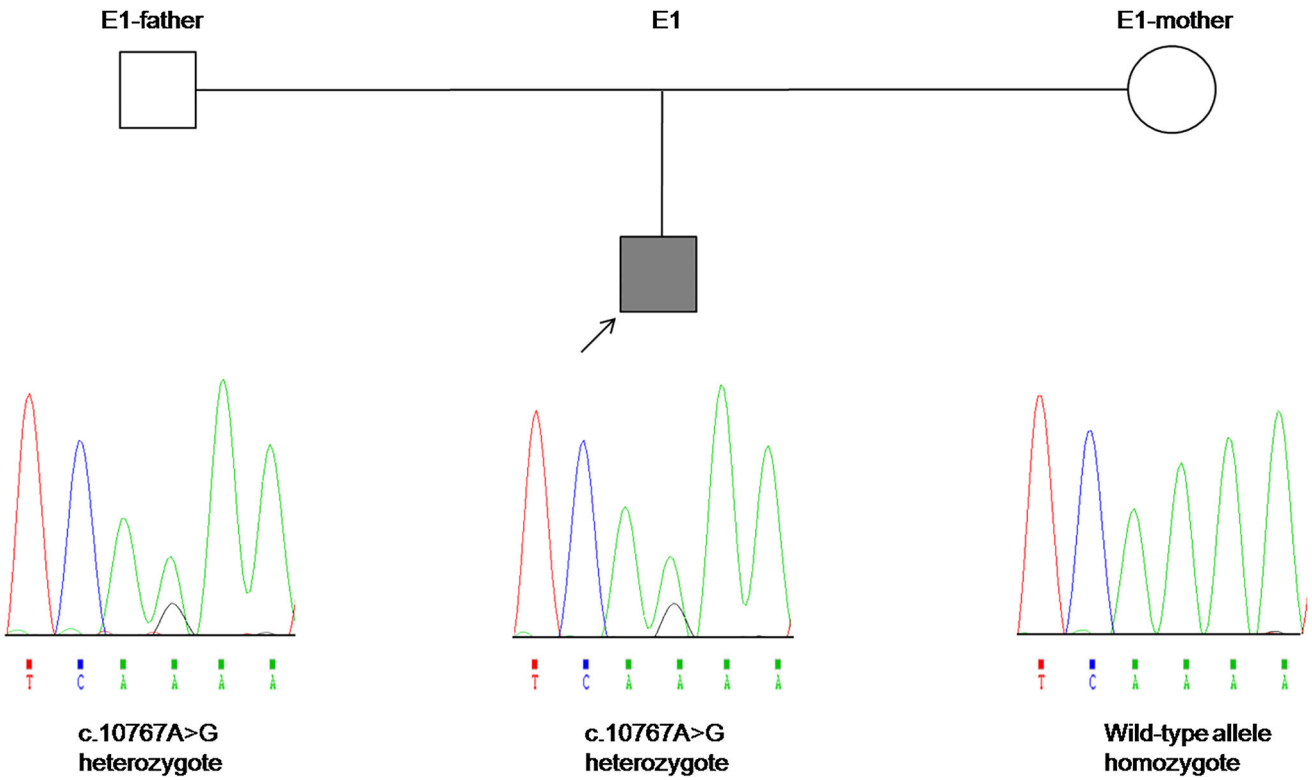

Figure 2. Pedigree of the family E1 and Sanger sequencing results of *DNAH5* exon 63.

analysis of exons 34, 50, 76 and 77 for all patients have shown two single-stranded DNA bands similar to the control (wild-type) sample (figure 1). However, the screening of exon 63 had shown a heterozygous profile consisting of four different single-stranded DNA bands in only one patient. The same PCR-SSCP profile has also been found in the patient's father. The DNA sequencing revealed a heterozygous variant (NM_001369.2: c.10767A>G) carried by the patient and his father, confirming the SSCP profile (figure 2). This variant is novel and has not been reported in gnomAD, 1000 genomes or any other publicly available databases. *In silico* analysis of the variant using the online MaxEntScan and Human Splicing finder tools (available at: http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq_acc.html and http://www.umd.be/HSF/index.html, respectively) have shown that this variant activates a cryptic acceptor splicing site (MaxEntScan score 7.48 vs −1.27) and potentially alters the splicing of *DNAH5* exon 63.

Discussion

PCD is an autosomal recessive and genetically heterogeneous disease of motile cilia. According to the literature, *DNAH5* is the most frequently mutated gene in PCD patients and a set of five exons in this gene have been reported as hot spots (Hornef *et al*. 2006; Bustamante-Marin *et al*. 2019). Therefore, the objective of this study was to screen the *DNAH5* hot spot exons for the first time to identify the deleterious mutations in a group of clinically diagnosed Tunisian PCD patients.

According to our results, no deleterious mutations were found in any of the hot spot exons of the patients. However, a novel heterozygous variant (c.10767A>G) in exon 63 was identified in only one patient and his father (figures 1 and 2). This variant activates a cryptic acceptor splicing site which potentially alters the splicing of exon 63 (MaxEntScan score 7.48 vs –1.27) and results in a frameshift mutation (p.(Gly3519Glufs*16)). However, the canonical splicing site of exon 63 has a relatively higher score than the cryptic site (12.89 vs 7.48). Therefore, the deleterious effect of the variant remains uncertain. Moreover, sequencing the rest of the *DNAH5* exons is necessary to search for a second mutation and confirm the diagnosis of PCD in this patient.

The study of Hornef and coworkers (Hornef *et al.* 2006) was the first to report a clustering of mutations within five exons (34, 50, 63, 76 and 77) of *DNAH5* gene which they considered as hot spots. Among 44 different independent alleles, they identified 25 mutations in hot spot exons (57%) (Hornef *et al.* 2006). Moreover, the only mutation of exon 63 identified in previous studies (c.10815del), is a founder mutation (Hornef *et al.* 2006).

The same exons were investigated by Djakow and coworkers (Djakow *et al.* 2012) and they have reported mutations in the hot spot exons in 12.5% (6/48) of independent alleles in a group of Czech PCD patients (Djakow *et al.* 2012).

This discrepancy in results may be attributed to the different geographical origins and ethnicities. In fact, the mutations of *DNAH5* hot spot exons were identified, mainly in Caucasian patients, whereas, the native background of the Tunisian population is Berber (Romdhane *et al.* 2012). Further, four mutations in the *DNHA5* hot spot exons (34, 63, 76 and 77) were reported in the literature as founder mutations in Caucasian populations (Hornef *et al.* 2006). Thus, *DNAH5* exons reported as hot spots in Caucasian populations should not be considered as a sequencing target in patients with different ethnical and geographical origins.

Moreover, 27 north African patients have been reported in literature between November 1999 and July 2019. These patients had mutations in 10 different genes (*CCDC39*, *CCDC40*, *RSPH3*, *RSPH1*, *DNAH9*, *GAS8*, *RSPH4A*, *CCNO*, *PIH1D3* and *DNAJB13*) and none of them had mutations in *DNHA5* (Merveille *et al.* 2011; Blanchon *et al.* 2012; Antony *et al.* 2013; Kott *et al.* 2013; Olbrich *et al.* 2015; Frommer *et al.* 2015; Jeanson *et al.* 2015, 2016; El Khouri *et al.* 2016; Amirav *et al.* 2016; Olcese *et al.* 2017; Fassad *et al.* 2018). This further highlights the specificity of the genetic background of North African populations.

In this study, we identified a novel heterozygous variant in *DNAH5* exon 63 in only one among 10 Tunisian patients clinically diagnosed with PCD. This variant does not confirm the diagnosis of PCD in the patient. Therefore, further molecular investigations, such as next-generation sequencing have to be performed to identify the underlying genetic factor of PCD in our group of patients.

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