



HAL
open science

Skewed X-chromosome inactivation drives the proportion of DNAAF6-defective airway motile cilia and variable expressivity in primary ciliary dyskinesia

Lucie Thomas, Laurence Cuisset, Jean-François Papon, Aline Tamalet, Isabelle Pin, Rola Abou Taam, Catherine Faucon, Guy Montantin, Sylvie Tissier, Philippe Duquesnoy, et al.

► To cite this version:

Lucie Thomas, Laurence Cuisset, Jean-François Papon, Aline Tamalet, Isabelle Pin, et al.. Skewed X-chromosome inactivation drives the proportion of DNAAF6-defective airway motile cilia and variable expressivity in primary ciliary dyskinesia. The European Society of Human Genetics, Jun 2024, Berlin (DE), Germany. inserm-04614713

HAL Id: inserm-04614713

<https://inserm.hal.science/inserm-04614713v1>

Submitted on 17 Jun 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Skewed X-chromosome inactivation drives the proportion of *DNAAF6*-defective airway motile cilia and variable expressivity in primary ciliary dyskinesia

P05.004.D

Lucie Thomas¹, Laurence Cuisset², Jean-François Papon^{3,4}, Aline Tamalet⁵, Isabelle Pin⁶, Rola Abou Taam⁷, Catherine Faucon⁸, Guy Montantin⁹, Sylvie Tissier⁹, Philippe Duquesnoy¹, Florence Dastot-Le Moal⁹, Bruno Copin⁹, Nathalie Carion², Bruno Louis³, Sandra Chantot-Bastarud^{1,10}, Jean-Pierre Siffroi^{1,10}, Rana Mitri⁸, André Coste^{3,11}, Estelle Escudier^{1,9}, Guillaume Thouvenin⁵, Serge Amselem^{1,9} and Marie Legendre^{1,9}

¹Childhood Genetic Diseases, Sorbonne Université, Inserm, Hôpital Armand-Trousseau, Paris, F-75012, France ²Service de Médecine Génomique, Assistance Publique Hôpitaux de Paris (AP-HP), Université de Paris, Hôpital Cochin, Paris, F-75014, France ³Service d'Oto-Rhino-Laryngologie et de Chirurgie Cervico-Faciale, AP-HP, Hôpital Bicêtre, Le Kremlin-Bicêtre, F-94270, France ⁴Institut Mondor de Recherche Biomédicale, Université Paris-Est Créteil, Inserm U955, CNRS ERL7240, Hôpital Henri-Mondor, Créteil, F-94010, France ⁵Département de Pneumologie Pédiatrique, Centre National de Référence des Maladies Respiratoires Rares RespiRare, AP-HP, Sorbonne Université, Hôpital Armand-Trousseau Hospital, Paris, F-75012, France ⁶Pédiatrie, CHU Grenoble Alpes, Grenoble, F-38500, France ⁷Service de Pneumologie et Allergologie Pédiatriques, AP-HP, Hôpital Necker-Enfants Malades, Paris, F-75015, France ⁸Service d'Anatomopathologie, Laboratoire de Microscopie Electronique, Centre Hospitalier Intercommunal de Créteil, Créteil, F-94000, France ⁹Génétique moléculaire, AP-HP, Hôpital Armand-Trousseau, Paris, F-75012, Paris ¹⁰Génétique chromosomique, AP-HP, Hôpital Trousseau, Paris, F-75012, France ¹¹Service d'ORL et de Chirurgie Cervico-Faciale, AP-HP, Hôpital Henri-Mondor, Centre Hospitalier Intercommunal de Créteil, Créteil, F-94000, France

Background

Cilia and flagella are evolutionarily conserved organelles whose motility relies on the outer and inner dynein arm complexes (ODAs/IDAs). Defects in ODAs/IDAs result in primary ciliary dyskinesia (PCD), a rare disease characterized by recurrent airway infections, male infertility and *situs inversus* (in 50% of cases). To date, about 50 genes have been implicated in PCD, usually following an autosomal-recessive pattern.

DNAAF6 is involved in the assembly of the dynein arm complexes. *DNAAF6* pathogenic variations were considered to cause an X-linked recessive PCD, with carrier mothers supposed to be asymptomatic.

Purpose : Our objective was to assess the possible clinical and ciliary consequences of allelic specificity of X-chromosome inactivation (XCI) in 6 mothers of PCD patients carrying *DNAAF6* pathogenic variations.

Airway phenotypes of mothers heterozygous for a *DNAAF6* pathogenic molecular defect

Mother ID number Age range (yr) at the time of study	Genotype	Respiratory-symptom onset (age)	Primary ciliary dyskinesia-related investigations			Airway disease	
			nNO ¹ (μL/min)	HSVA (% motile/immotile ciliated tufts) (CBF)	TEM Abnormal cilia (%)	Upper	Lower
M1 DCP1339 45-50	c.[3.27-Mb del],[=] p.[0],[=]	-	ND	ND	ND	None	None
M2 8822GM0145 35-40	c.[118-kb del],[=] p.[0],[=]	-	ND	ND	ND	None	None
M3 16GM00624 45-50	c.[3.73-Mb del],[=] p.[0],[=]	Adult (38 yr)	Not evaluated	Motile: 83% (8.1 Hz) Immotile: 17%	Not assessable (no ciliated cells)	Intermittent nasal obstruction	Some bronchitis episodes from 38 years old
M4 DCP1748 35-40	c.[1.93-Mb del],[=] p.[0],[=]	Childhood (10 yr)	159	Motile: 77% (4.5 Hz) Immotile: 23%	Absence of both dynein arms (3%)	Sinusitis during childhood	Wet cough during childhood; none during adulthood
M5 DCP1848 40-45	c.[511C>T],[=] p.[Gln171*],[=]	Childhood (5 yr)	248	Motile: 50% (15.5 Hz) Immotile: 50%	Absence of both dynein arms (80%)	Chronic rhinosinusitis; otitis only during childhood Partial sinus opacification	Some bronchitis episodes; one episode of pneumonia (at 39 yr)
M6 DCP1994 45-50	c.[397G>T],[=] p.[Asp133Tyr],[=]	Childhood (6 yr)	Normal	Motile* (9.5 Hz) Immotile*	Absence of both dynein arms (45%)	Serous otitis; chronic rhinosinusitis with surgery (at 6 & 20 yr); nasal polyposis Total sinus opacification Purulent secretions	Several episodes of pneumonia; chronic bronchitis; exacerbations with intermittent <i>P. aeruginosa</i> Bronchiectasis involving the left inferior lobe

nNO: nasal nitric oxide; HSVA: high-speed videomicroscopy analysis; CBF: ciliary-beat frequency; TEM: transmission electron microscopy; ND: not done.

*ND in PCD patients <77 μL/min (2).

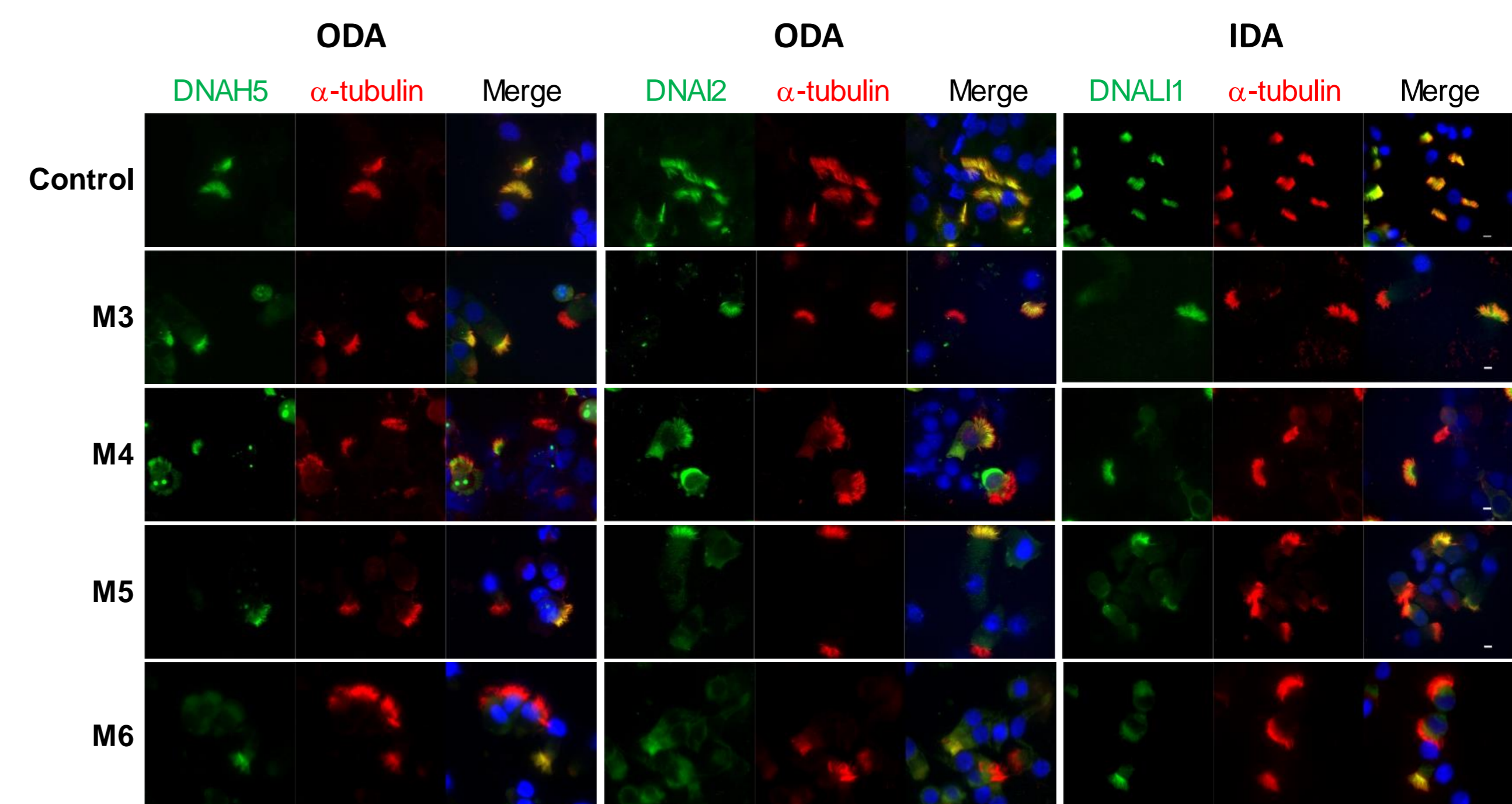
*Examined visually but % of immotile ciliated cells not evaluated

□ No symptoms □ Mild phenotype □ Moderate phenotype □ Severe phenotype

- Clinical phenotypes differed, ranging from no respiratory symptoms (mothers M1 and M2) to variable disease severities (M3, M4, M5, M6)
- M6's greater symptom severity is close to typical PCD
- None of the six mothers had *situs inversus*

Impact of heterozygous *DNAAF6* mutations on dynein arm-specific proteins in airway epithelial cells (AECs)

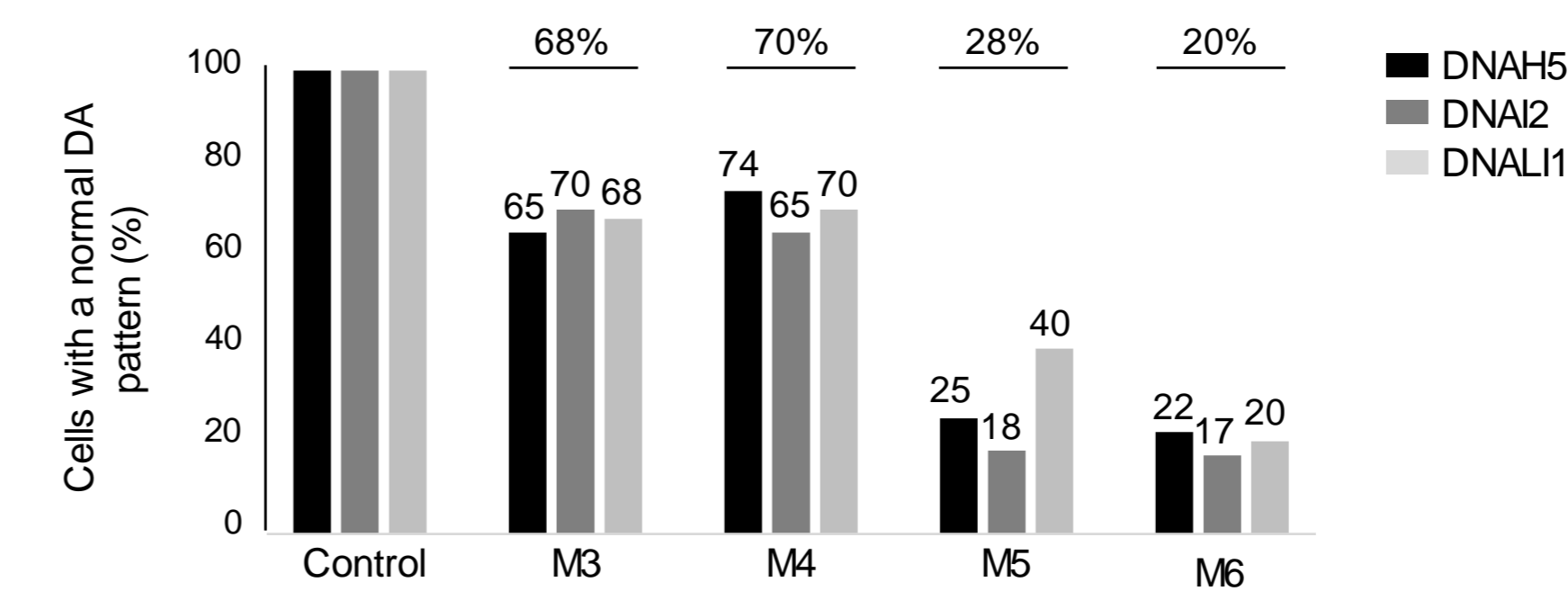
Functional mosaicism in airway multiciliated cells



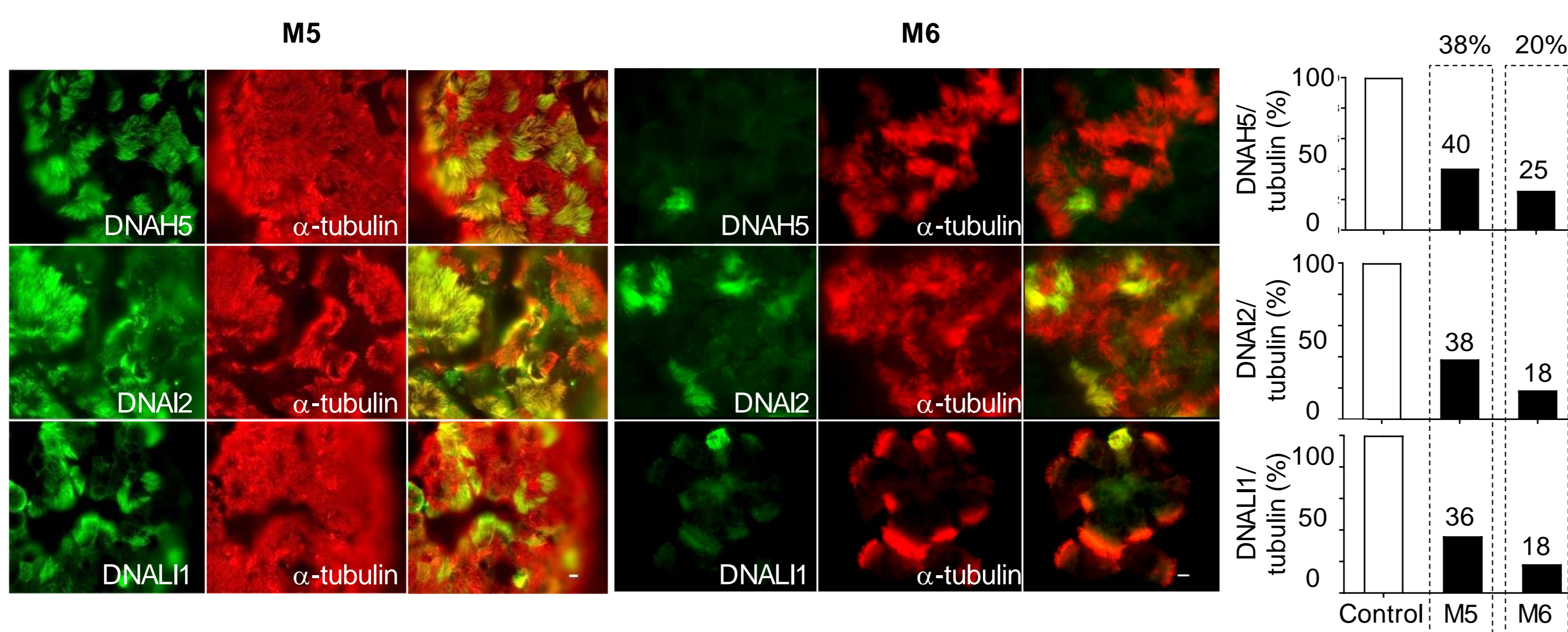
→ Normal ciliated cells expressing the normal *DNAAF6* allele show dynein arm components along the axoneme (DNAH5 and DNAI2 in ODA; DNALI1 in IDA)

→ Abnormal cells expressing the mutated *DNAAF6* allele lack the ODA and IDA markers

Proportion of normal AECs

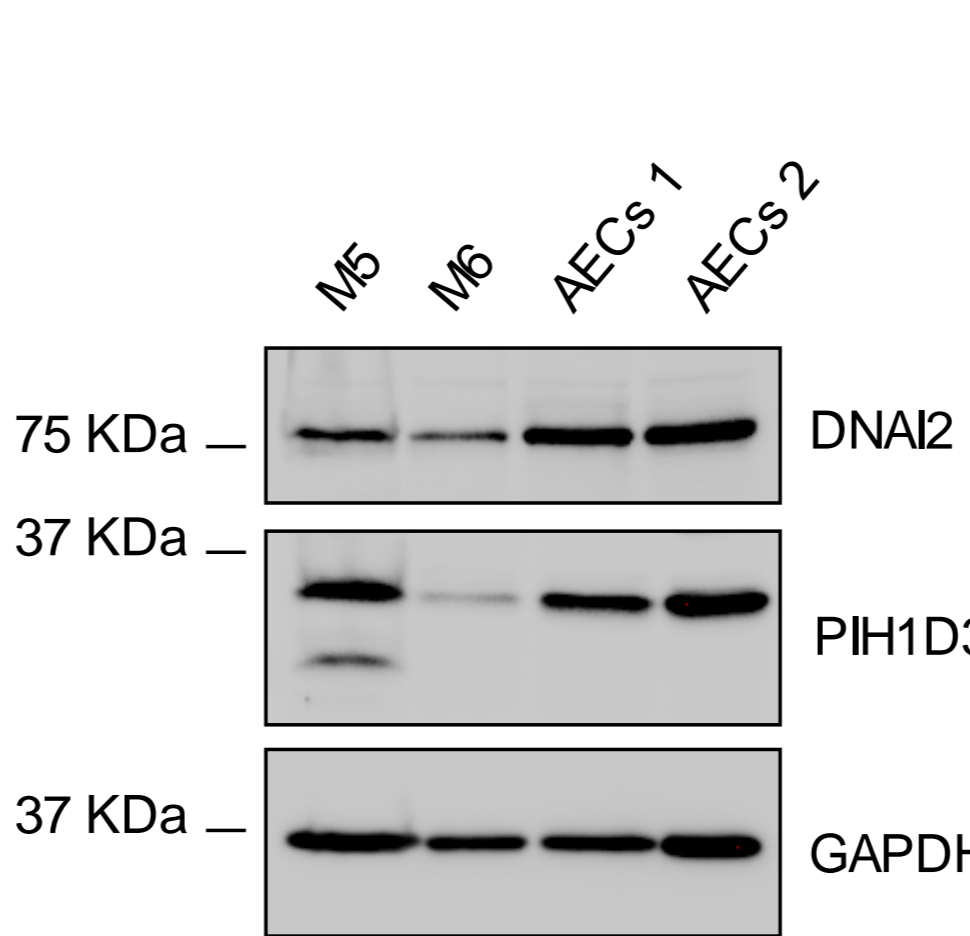


Deep phenotyping of air-liquid interface (ALI)-cultured AECs from mothers M5 and M6

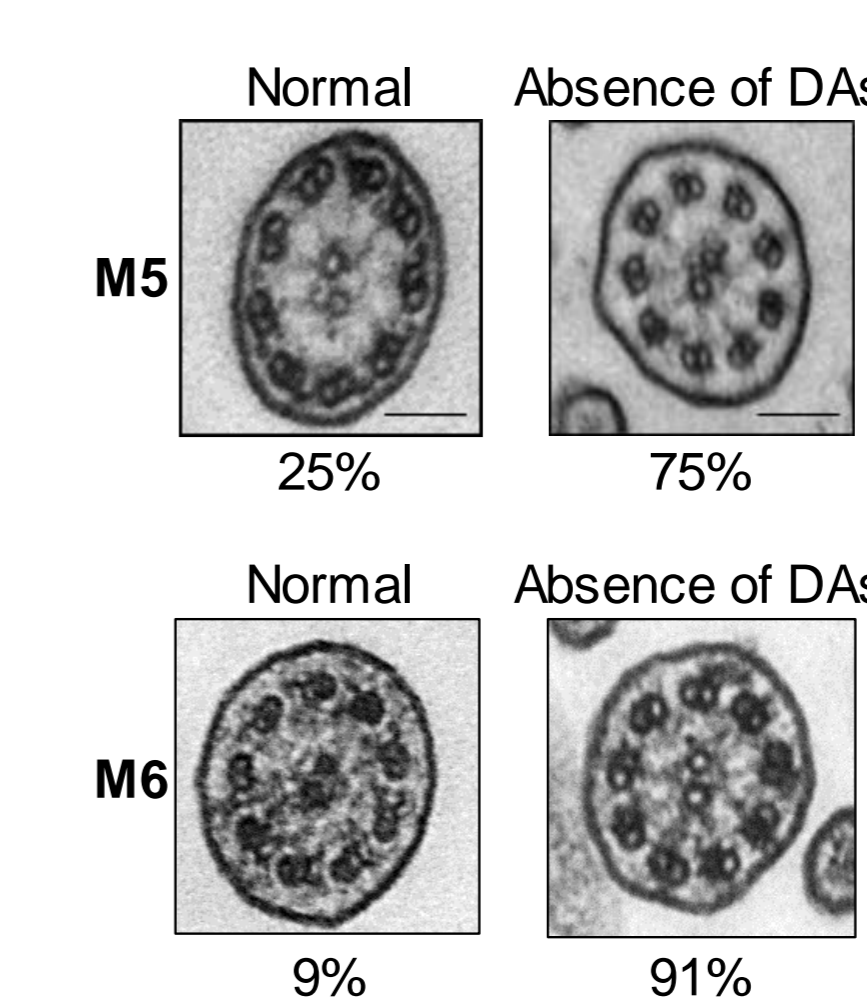


- Immunolabelling showed patches of AECs expressing either the normal or the mutated *DNAAF6* allele (DNAH5, DNAI2 and DNALI1: markers of dynein arms in ciliated cells)

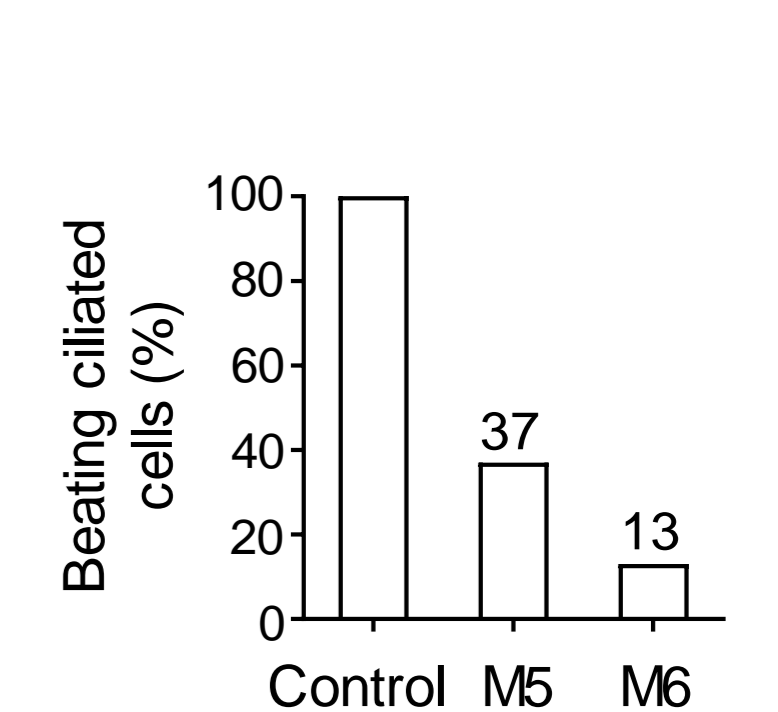
Western blot



Transmission electron microscopy (TEM)

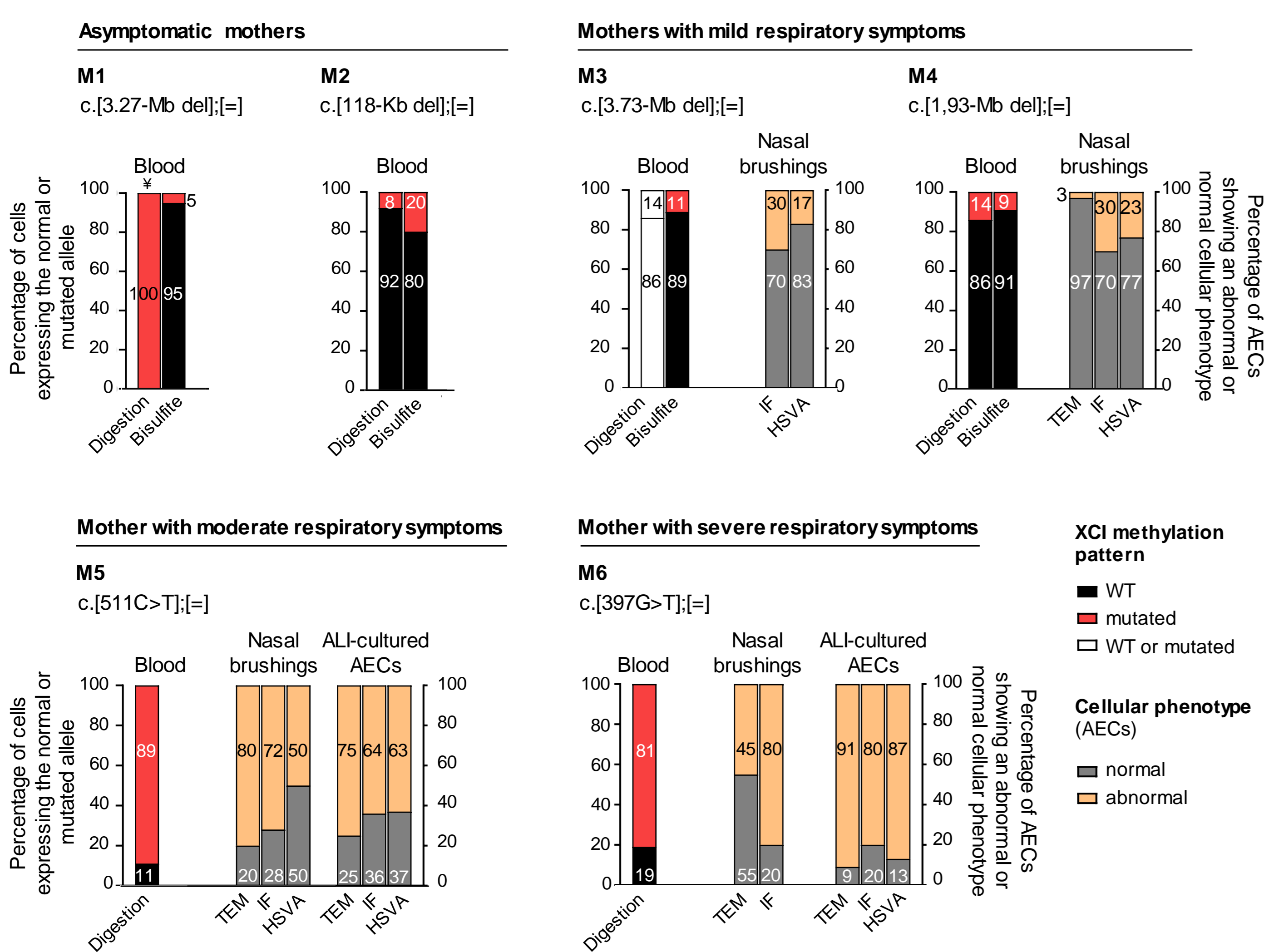


High Speed Videomicroscopy



- Cellular mosaicism : ≈35% of normal ciliated cells in M5 (moderate phenotype) and ≈15% of normal cells in M6 (severe phenotype)

X-chromosome inactivation (XCI) patterns vs clinical and cellular phenotypes



XCI measured in blood and/or AECs by two methods:

- 1) methylation-sensitive digestion at the *HUMARA* locus
- 2) bisulfite conversion of cytosine at the *DNAAF6* locus (only feasible for whole gene deletions)

▪ The digestion method revealed limitations due to the distance between *HUMARA* and *DNAAF6* and probable recombination events:

- failure to phase the XCI status with the *DNAAF6* allele in M3 (white histogram)

- inconsistent result in M1 (¥).

• The bisulfite method effectively addressed the aforementioned issues.

- XCI skewing among mothers heterozygous for mutated *DNAAF6*:
 - favouring the normal allele expression in M1, M2, M3 and M4 (asymptomatic & mild phenotypes)
 - favouring the mutated allele in M5 and M6 (moderate and severe phenotypes)

Conclusion and perspectives

- The clinical phenotypes of the mothers are reflective of their XCI pattern.
- In the airways of symptomatic mothers, there is a coexistence of normal cells and cells with immotile cilia lacking dynein arms, with a ratio closely reflecting their XCI pattern.
- The bisulfite-conversion method, which is specific of the *DNAAF6* locus, is a more reliable than the methylation-sensitive digestion technique.
- The preferential inactivation of X chromosomes harbouring large multigenic deletions is protective in mothers: asymptomatic and mild phenotypes correspond to large multigenic deletions (M1, M2, M3 and M4). Conversely, M5 and M6 who carry point mutations, exhibited moderate and severe phenotypes respectively.
- It is crucial to provide testing for all female relatives of male PCD patients with a *DNAAF6* defect, and potentially to females consulting for mild chronic respiratory symptoms.
- In the 6 mothers, the nodal flow, which results from the beating of nodal cilia, may have been sufficient to result in normal organ lateralisation.
- Our findings also indicate that approximately 30% of normal ciliated airway cells were sufficient to prevent severe PCD, a result that paves the way for gene therapy.

These data have been published in Thomas et al., J Med Genet, 2024

The authors have no conflict of interest.