



University of Dundee

## **C11orf70 Mutations Disrupting the Intraflagellar Transport-Dependent Assembly of Multiple Axonemal Dyneins Cause Primary Ciliary Dyskinesia**

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Homo	1	MATGELGF-----IGGYYFRFLPQKTFQSLSSKEITSRRLRQWSMLGRIKAQAFGFDQ
Bos	1	MAAGEPGD-----ICVYSFRFLPQKTFQSLSTPQTTSRRLRQWSMLGRIEAQAFGFDQ
Mus	1	MAAGEPRD-----GGYYFRFLPHHTFSSLSAREITSRRLRQWSMLGRIQAQAFSFDQ
Rattus	1	MATGEPRI-----RGGYYFRFLPQKTFSSLSAREITNRLRQWSMLGRIQAQAFGFDQ
Xenopus	1	MSAGSFST-----EAAKFSFSPILNKTFFGFLINRDTRELLMKWSMNGRITAQAARIDE
Danio	1	MVTTTIGIMAEKLSFEQTEFNENLSTKSENFQEDPKTSRLIMKWSMLGRIITAQAFNFDQ
Chlamydomonas	1	MTAF-----VPSLPSSTSAINDAYVKSQITKWDIIRNIRCVAVRYTK
Paramecium	1	MQIESDNQ-----VTNQSISFRCE-NIILDDKFMELIQKWIQHSIKVSTLEFDI

Homo	53	TFQSYRKDDFVMAFFKDPNVIPNLKLLSSSGQWITLGTEVKKIEAINVPCTQLSMSFFH
Bos	53	TFQAYRKDDFVTAFKDPNVIPNLKLLSESSGQWITLGTEVKKIEAINVPCTQLSMSFFN
Mus	53	TFQPYQKDDFVMAFFKDPNVIPNLQLLSSSGQWITLGSEVKKIEAINVPCTQLSMSFFQ
Rattus	53	TFQPYQKDDFVTAFKDPNVIPHLQLLASSGQWITLGTEVKKIEAINVPCTQLSMSFFQ
Xenopus	54	CFQPYQKDDFVMAFFQDEVDVISHLKIYSENSGQWITLGTQVKKVIVQETLCSQLSMSIFD
Danio	61	SFQPYRSNDEAWNFQDEPCVKHNLNVID-PIGSWTRLG-DITHNVEVVPCTKIVSDIFD
Chlamydomonas	43	YYHKLGGQLIADLFEDEKVVQEAFOVIRK-GGAWGQLGGPVTKVVDATLLASSLTRMDIFD
Paramecium	52	KFDHLIPNQFLLDLLENSKDVGRSLHYVVSF-K-C----NVLISQIKFQPLTCKSKRDIKDFD

Homo	113	RLYDE--DIVRSGHIVKCLDSFCDFLISDELRVLLVEDSEKYEIIFSQPDREEFFLFCI
Bos	113	RLYDE--AIVRDNGYIVKCLDSFCDFLISDELKRVLLVEDSEKYEIVFSQPEREEFFLFCI
Mus	113	RLYDE--NIVRSGHIVKCLDSFCDFLISDELKRVLLMEDSEKYEIVFSQPEREEFFLFCI
Rattus	113	RLYDE--NIVRSGHIVKCLDSFCDFLISDELKRVLLMEDSEKYEIVFSQPEREEFFLFCI
Xenopus	114	CLYSE--GIVRSGHIVKCLDEYLDLDTISDELKRVLLDDECKHIVFSQSDREQFLLELI
Danio	119	PIYSN--GIIIRPSGHIVKCYHEIYDPD---DELRLVLEADSEYHHTISPSDROEFLERL
Chlamydomonas	102	KLITETSPETIVRSNGDICKGMEDNREGHQVSDQRLRELLVEESEAHAIFSEABROETLRL
Paramecium	106	KLITED--KIVV-KGHIKQCFEPEQFNIQTADELKRVLLVEDSEKVCVENEADROETLEKLI

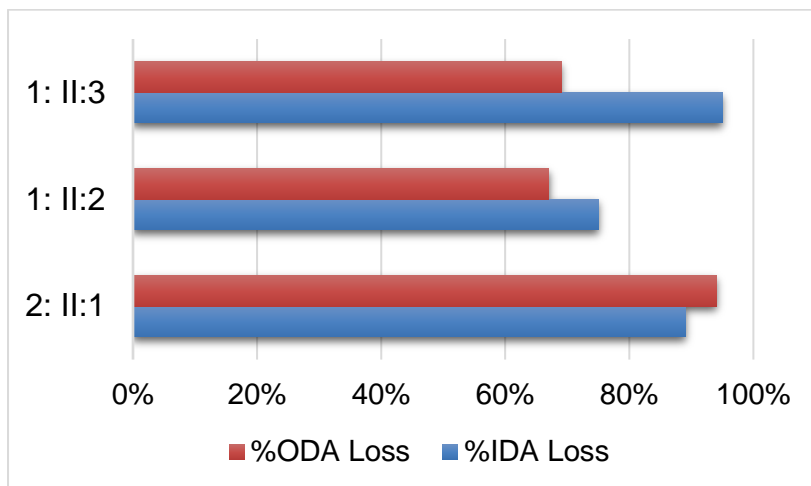
  

Homo	171	FKHLCLGGALCQYEDVISPYLETTKLIYKDLVSVRKNFQTKKIQITSSVFKVAYDS-AG
Bos	171	FKHLCLGGALCQYEDVINPYLETTKLIYKDLVSVRKNFQTKKIQITSSIFKVAYDS-VG
Mus	171	FKHLCLGGSILCQYEDVIKPYLETAKLIYKDLVSVRKHERTKEIQITSSVFKVKAYDS-VG
Rattus	171	FKHLCLGGSILCQYEDVIKPYLETAKLIYKDLVSVRKHERTKEIQITSSVFKVKAYDS-LG
Xenopus	172	FKHLCLGGALCQYEDTIGPYLETTKSIYKDLISVOKDEETKQIRIISIVFKVAYDE-NG
Danio	174	FKHIVLGGELCQYEDVIDPYLETVKIYKDLVSVQKDTDTKEINVVITVLKVSAYDH-SG
Chlamydomonas	162	FEHIVLGGALCQYEDKVEPEYVETSKRIYKELVCAQKDEATGKIVQTVSAVYKINSIQGDSG
Paramecium	163	FQIIVLGGQLCQYEDETQAYLDWTKYLYKNTVNARKYADKDEIYIDSYAYDIRKL-----

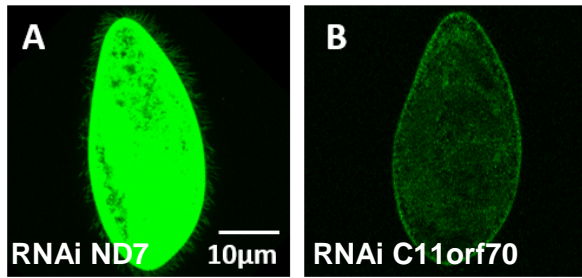
  

Homo	230	MCYPSAKNHEQTFSYFVDPPIRHHVLYHCYGVGDMSS--
Bos	230	VCYPSIKSHEQTFSYFVDPPIKRHHVLYHCYGVGGEVSS--
Mus	230	VCYPSPKSHEQTFSYFVDPPIKRHVNVLYHCYGVGHVA--
Rattus	230	VCYPSPKSHEQTFSYFVDPPIKRHVNVLYHCYGVGEMA--
Xenopus	231	MCYPSGPHQQTFAYLVDPLKRHVNVLYHCEGGGA-F--
Danio	233	LCYPSATNPKQTEAYLCTDPCKRHVNVLYHSEFGIGVFSGN
Chlamydomonas	222	PLELYPSRSRQNFQYAAVDPVRRIVKLYHAYVPYW----
Paramecium	218	-ENSYSSHPQNVMYVNVNESLRIVNLEENQMLKVV----

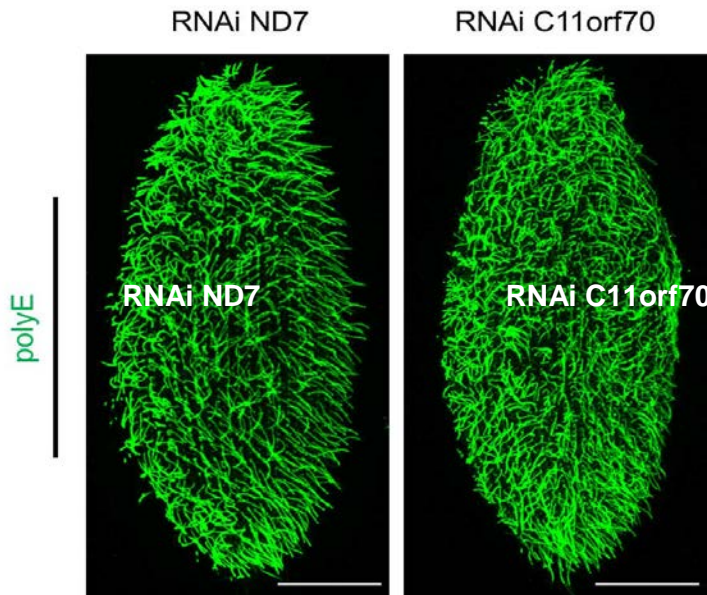
**Figure S1. Cross species protein alignment for C11orf70**



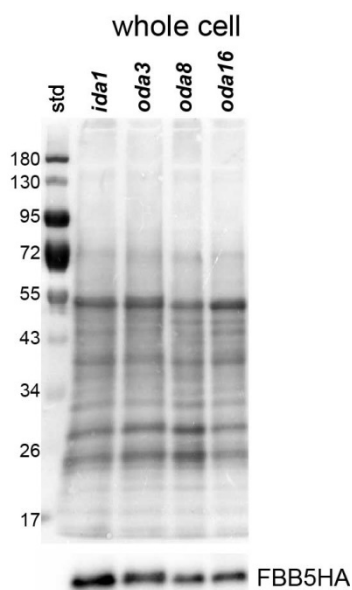
**Figure S2. Quantification of TEM analysis of outer and inner dynein arm loss in affected individuals with C11orf70 mutations**



**Figure S3. Successful RNAi ablation of *C11orf70* shown in *Paramecium* transformed with GFP-tagged *C11orf70*.** *ND7*- (control) and *C11orf70*- silenced *Paramecia* were fixed and visualised for *C11orf70*-GFP protein expression after 72h of RNAi. Scale bars, 10 µm.



**Figure S4. Depletion of *C11orf70* in *Paramecium* does not affect cilia number and length.** *ND7* (control) and *C11orf70* silenced *Paramecia* were fixed and stained for cilia after 72h of RNAi. Cilia were stained using anti - polyglutamylated tubulin antibodies to indicate the cilia (PolyE). Scale bars, 20 µm.



**Figure S5. *Chlamydomonas* FBB5 is expressed at similar levels in a number of mutant *Chlamydomonas* strains.** Amido black stained gels shows equivalent protein in strains with mutations in proteins shown in Figure 5, main text.

Primer ID	(5'- Sequence -3')
<b>Sanger sequencing for c.776A&gt;G mutation</b>	
Fwd	GATCCTATCAGGCGTCACCTT
Rev	ATCCCCGGTTAGTATTCCACAC
<b>Sanger sequencing for c.154C&gt;T mutation</b>	
Fwd	GCCAGCCCCCAGATGATTT
Rev	GAGACCCAGGGAAGCTCTCCG
<b>Sanger sequencing for c.361C&gt;T mutation</b>	
Fwd	GTTCTTGACACAGCTTTCAA
Rev	TCCTGAAATAAAATTCCACTGCGA
<b>q(RT-PCR) for expression profiling of C11orf70 in ALI culture</b>	
C11orf70-Fwd	TGGGTGGCTACTACTTCAGGT
C11orf70-Rev	GCCTTGATTCTGCCAGCAT
DNAH5-Fwd	TGCAGATGCCATGGTTCCT
DNAH5-Rev	ATGAAGCCAACCTCGTCAGG
GAPDH-Fwd	TGCACCACCAACTGCTTAGC
GAPDH-Rev	GGCATGGACTGTGGTCATGAG
<b>561bp segment of <i>Paramecium</i> GSPATG00011350001 for RNAi design</b>	
Fwd	TAGAAGTAGTATGTAAATTGAATCAGACAATTAAGTTAC
Rev	TTTTTCTCGAGCTTTGTCCAATCTAAATAGGCTTG
<b>q(RT-PCR) for GSPATG00011350001 knockdown in <i>Paramecium</i></b>	
GSPATG00011350001-F	TGCTCGAAAATATGCAGATAAGGA
GSPATG00011350001-R	TGATGGGTTCAACGACAT
GAPDH-Fwd	GAGAGCCGGAAGAGCTGCTA
GAPDH-Rev	TGGTGGAAGCTCTGAAGGCCATA
<b>Amplification of <i>Paramecium</i> GSPATG00011350001 (C11orf70) for GFP expression vector cloning</b>	
Fwd	TAGAAGTAGTATGTAAATTGAATCAGACAATTAAGTTAC
Rev	TTTTTCTCGAGTCTCCTCCCATACTTTTAACCATTAGTTTTCT
<b>Amplification of <i>Paramecium</i> GSPATG00024708001 (IFT46) for GFP expression vector cloning</b>	
Regulator-Fwd	ATACTTGGATCCTATTTAATATAATTAATAAATACTGTTATTC
Regulator-Rev	GACAAAACAATGATGAAATTTAAGGCATGCAAGTAT
GSPATG00024708001-F	ATACTTGGTACCTGAATATTCAATAATATAACATTATTTTC
GSPATG00024708001-R	GGAATATTTATTATAATTATTA

**Table S1.**  
Primers

**used in the study**

Antigen	Antibody	Host species	Source	Application
DNAH5	HPA037470	Rabbit	Sigma-Aldrich	IF (1:800)
DNAL1	HPA053129	Rabbit	Sigma-Aldrich	IF (1:200)
RSPH4A	HPA031196	Rabbit	Sigma-Aldrich	IF (1:400)
GAS8	HPA041311	Rabbit	Sigma-Aldrich	IF (1:500)
Acetylated Tubulin	T7451	Mouse	Sigma-Aldrich	IF (1:500)
Acetylated Tubulin	YF488	Mouse	Proteintech	IF (1:500)
Polyglutamylated Tubulin	PolyE	Rabbit	A gift from J. Cohen	IF (1:500)
GFP	IgG	Rabbit	Interchim, France	IF (1:500)
HA	McAb 3F10	Rat	Roche, Indianapolis	IB (1:1000)
<i>Chlamydomonas</i> IFT46C	α-IFT46C404	Guinea Pig	A gift from G. Witman	IB (1:10000)
<i>Chlamydomonas</i> IFT46N	α-IFT46-17601	Rabbit	A gift from H. Qin	IB (1:5000)
<i>Chlamydomonas</i> IFT81	81.3	Mouse	A gift from D. Cole	IB (1:10000)
<i>Chlamydomonas</i> IFT139	139.1	Mouse	A gift from D. Cole	IB (1:10000)
<i>Chlamydomonas</i> IC2	C11.4	Mouse	DRM lab	IB (1:2000)
<i>Chlamydomonas</i> ODA16	α-ODA16-927	Rabbit	DRM lab	IB (1:100)

**Table S2: Primary antibodies used in the study**

Antibody	Host Species	Source	Application
Alexa 488, 594 – conjugated anti-rabbit IgG (H+L)	Goat	Invitrogen (Molecular Probes)	IF (1:1000)
Alexa 488, 594 – conjugated Anti-mouse IgG1	Goat	Invitrogen (Molecular Probes)	IF (1:1000)
HRP-conjugated Anti-mouse IgG	Goat	BioRad	IB (1:2000)
HRP-conjugated Anti-rabbit IgG	Goat	Sigma-Aldrich	IB (1:2000)
HRP-conjugated Anti-guinea Pig IgG	Goat	Rockland	IB (1:1000)
HRP-conjugated Anti-rat IgG	Goat	Sigma-Aldrich	IB (1:5000)

**Table S3: Secondary antibodies used in the study**