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Abstract:
Cilia are membrane bound, microtubule-based organelles involved in cellular activities ranging from sensory perception to motility and fluid movement. These cilia project from almost every cell type in the body. Defects in cilia proteins cause the autosomal recessive developmental disorders Nephronophthisis (NPHP), Joubert Syndrome (JBTS), and Meckel-Gruber Syndrome (MKS) in humans; however, in most cases, the disrupted genes have not yet been identified. Many of the known NPHP, JBTS, and MKS genes are conserved in the nematode Caenorhabiditis elegans, raising the possibility that this simple model organism can be used to understand the human disorders. Single mutations in NPHP or MKS genes in C. elegans have minimal affects on ciliogenesis; however, we have found that combinations of NPHP and MKS gene mutations alter cilia formation and positioning. Here, this redundant requirement of NPHP and MKS genes was utilized in a forward EMS mutagenesis screen in C. elegans to identify novel candidate genes involved in human NPHP or MKS. Mutagenesis was performed on nphp-4(tm925) mutant worms and progeny lacking properly formed cilia were isolated for analysis. Genes disrupted in this screen are being identified by whole genome sequencing and confirmed by transgenic rescue experiments. Ultimately, NPHP and MKS families will be screened for potential mutations in the human homologs of genes identified from this screen.

Introduction:
The autosomally recessive Nephronophthisis (NPHP)-associated disorders are heterogenic and affect a variety of organs. Also termed ciliopathies, these genetic disorders result from mutations affecting proteins of largely unknown function that localize to the cilia or base of the cilia (basal bodies). In NPHP, cysts will form within the corticomedullary border of the kidney. These symptoms tend to be isolated to the kidneys with renal interstitial infiltration in addition to fibrosis, and basement membrane disruption along with tubular atrophy. The most severe NPHP-related ciliopathy is known as Meckel-Gruber Syndrome (MKS). MKS patients traditionally do not live past birth, or are naturally aborted earlier. The additional symptoms of this autosomal recessive lethal disorder are central nervous system malformations, occipital encephalocele, post-axial polydactyly, bowing limbs, severe heart malformations, and hepatic developmental defects. There is extensive genetic overlap between MKS and NPHP with distinct mutations identified in shared genes. This indicates that disease severity, and thus, clinical diagnosis, is influenced by which gene is affected, the nature of the mutation in that gene, and the genetic background of the patient. Unfortunately, the causative lesion in most MKS and NPHP patients remains unidentified. Identifying the missing genes involved in these disorders is critical to ultimately understanding the cellular and molecular basis of the disease, and in turn, developing possible genetic therapeutic strategies. [6]

A large number of NPHP and MKS genes are conserved in the nematode *C. elegans* (Figures 1 and 2). Whereas humans have primary cilia extending from the majority of their cells, *C. elegans* only have sensory cilia extending from a subset of their neurons in the head (amphids) and tail (phasmids). The cilia have a basal body complex that anchors the cilium axoneme to the plasma membrane and cytoskeleton. The basal bodies function in the assembly of proteins that are involved in intraflagellar transport (IFT) in addition to initializing ciliogenesis. IFT is a critical component of cilia formation and it mediates the trafficking of proteins along the cilia axoneme. When mutations occur within genes encoding the basal body and IFT components in mice, symptoms of the aforementioned ciliopathies (diseases associated with ciliary defects) will result. In contrast to the critical requirement of cilia for mammalian development, these cilia function primarily as sensory organs of the worm and are not essential for the viability of the organism. The nonessential nature of the sensory cilia, along with the genetic malleability of *C. elegans,* facilitates the analysis of interactions between the large number of NPHP and MKS gene homologs with relation to cilia structure and/or function. [4,6]

Biochemical and genetic analyses have indicated that MKS and MPHP proteins form two distinct but potentially interacting complexes at the base of the cilia. Solitary mutations in genes in one complex will not cause a visible defect in cilia morphology in *C. elegans*; there will only be a visible adverse effect when a combination of disruptions in both complexes exists. This combination causes a striking deactivation of cilia function to be induced. The disruption of cilia morphology in *mks;nphp* double mutant worms is most easily observed via a dye-filling assay in which the animals are exposed to a hydrophobic fluorescent dye. If cilia structure is normal, the dye is taken in through the cilia membrane and spreads throughout the sensory neurons. In the absence of properly formed cilia, the dye cannot stain the neurons. This phenomenon is referred to as dye-filling defective (Dyf) phenotype. Any combination of mutations in the currently known *mks* genes with the *nphp-4(tm925)* mutation results in the Dyf phenotype (Table 1, Figure 3). Based on this phenomenon,

we hypothesized that novel candidate *mks* genes could be targeted in a mutagenesis screen for Dyf isolates in the context of the *nphp-4(tm925)* mutation. Once the successful mutants are identified from the *C. elegans* screen, a homolog in the human genome might then be identified and screened in ciliopathy patients in whom a causative mutation has not been found. [6,7]

Procedure / Results:

An ethyl methane sulfate (EMS) mutagenesis screen was performed on *nphp-4(tm925)* mutant worms to introduce additional mutations into the genome with the intent to target novel genes that affect cilia morphology in the context of the *nphp-4* mutation. The EMS mutagenesis was used because of its ability to generate nonsense mutant alleles by implementing deletions or rearrangements in the nucleotide base-pairs.

From this EMS screen, ~170 mutant strains with a Dyf phenotype were initially obtained. This phenotype is not observed in any of the non-mutagenized *nphp-4(tm925)* worms. Each strain was then backcrossed with N2 Bristol males (wild-type) to eliminate any background mutations in the genome and to determine the dependency of the Dyf phenotype on the *nphp-4(tm925)* allele. Once they laid F1 progeny, the mutagenized parents were removed from the plates and the hermaphrodite progeny were dye-filled. Worms with a wild-type phenotype that were able to uptake dye (dye-fill) were selected. These were then allowed to self-fertilize. The parents were then removed and from the F2 progeny, dye-filling-defective (Dyf) worms were selected provided the collection of progeny exhibited a Mendelian ratio of 1 Dyf:15 wild-type (Figure 4). If the progeny had followed a ratio of 1 Dyf:3 non-Dyf, this would be indicative of a single gene recessive trait acting independent of the *nphp-4(tm925)* mutation. By having a ratio of 1 Dyf:15 non-Dyf, there is evidence that the genes are assorting independently from each other, and a homozygous recessive genotype for both traits is required for a Dyf phenotype. Again these progeny were allowed to self-mate. Their progeny were then dye-filled to verify the Dyf phenotype was transmitted to 100% of the progeny, indicative of a mutation to homozygosity. If hermaphrodite progeny possess a true homozygous recessive phenotype, they will only be able to produce homozygous recessive offspring. The outcrosses to the N2 wild type worms were repeated three times. Mutant strains that segregated with the 1:15 ratio were then genotyped to verify they were homozygous for the *nphp-4(tm925)* mutations. From the screen, we obtained strains 39.3, 91.3, and 5.3 showing a Dyf phenotype that was dependent on the *nphp-4(tm925)* mutation. The mutant strains' cilia was then imaged to verify the delocalization and non-functionality of the ciliary fibers (Figure 5). The outcrossed Dyf strains retaining the *nphp-4(tm925)* mutation were then mapped to a chromosome using SNP analysis.

For this project, I focused on lines 5 and 39 for further analysis. A SNP map, also referred to as bulk segregate analysis, was constructed to identify the locus of the mutations on the *C. elegans* genome. Briefly, the mutagenized strain was outcrossed with the Hawaiian (SNP variant) strain. SNPs variants between the Bristol N2 and Hawaiian strains were identified via a Polymerase Chain Reaction (PCR) across regions of each chromosome. Eight SNP variants on each chromosome were selected having different *DraI* restriction endonuclease digestion profiles which can be detected on gel electrophoresis analysis. For this analysis the *nphp-4(tm925)* novel gene mutagenized strain on the N2 Bristol background were crossed with the Hawaiian strain. All the progeny from this cross will have a random mix of SNP-*DraI* digest profiles. Subsequent progeny from the F1s of the N2/Hawaiian cross where the SNPs segregate randomly were then grouped according to the Dyf phenotype and SNP analysis was performed. N2 derived SNPs located near the new mutation will be enriched in the worms with the Dyf phenotype, while SNPs located distant from the mutation or on distinct chromosomes will have an equal N2 and Hawaiian ratio due to random segregation. When these reactions are run in an electrophoresis gel, the mutation can be narrowed down to a specific region on a chromosome. [3]

Briefly, from the F2 progeny ~50 worms that were Dyf and ~50 worms that were non-Dyf were selected to be lysed. The Dyf and non-Dyf worms were then added to two separate tubes, lysed and genomic DNA was isolated. SNP regions were amplified by PCR and the DNA was digested by a *DraI* restriction endonuclease. The digested DNA was separated by agarose gel electrophoresis. The gel was imaged to obtain the respective banding pattern for each strain.

The SNP mapping revealed a mutation on chromosome II for both strain 5 and strain 39. The mutation in *nphp-4(tm925)* on chromosome V was also evident in the SNP map (Figure 6). Together, the mapping data indicate the requirement of mutations in both genes to obtain a Dyf phenotype. Complementation analysis was then conducted by crossing lines 5 and 39 resulting in progeny that were all Dyf, thus indicating that the mutations in these lines are in the same gene. These results verify the phenomenon previously hypothesized that novel candidate genes, which could possibly be homologous to those undiscovered *mks* genes, may be identified through a mutagenesis screen with an *nphp(tm925)* already present. These results indicate that we have identified a mutation in a novel gene which functions along with NPHP4 to regulate cilia formation. Based on the previous data, we predict that this new gene will be a strong candidate in human MKS patients for which the underlying genetic cause is not yet known.

Future endeavors:

The next major goal is to identify the gene mutated in lines 5 and 39. For this we are using a Whole–Genome Sequencing approach. Deep-sequencing will be performed on mutant genomes of both line 5 and 39 followed by bio-informatic

Figure 1: Alignment of Homo sapiens NPHP4 with Caenorhabiditis elegans nphp-4

Figure 1 continued...

```
hsNPHP4          LRQGRPAVQASHELEVVATEYEQDNMVVSGDMLGFGRVKPIGVHSVVKGRLHLTLANVGH 829
ceNPHP-4         YRRGREAVQLFIQCPVVDTSLDTS--------------------SKAGAFLYMRVANIGF 747
                  *:** ***   :  ** *. : .                     * . . *:: :**:*.

hsNPHP4          PCEQKVRG---CSTLPPSRSRVISNDG-ASRFSGGSLLTTGSSRRKHVVQAQKLADVDSE 885
ceNPHP-4         PSGNTYDLSSSSSSLTTTRSNVNSGQGTVVRRLTSSIRLNEEGPHSYRIHAKPLPGNSGV 807
                 *. :.      .*:*..:**.* *.:* . *   .*:  . .. :.: ::*: *.. ..

hsNPHP4          LAAMLLTHARQGKGPQDVSRESDATRRRKLERMRSVRLQEAGGDLGRRGTSVLVRQSVRT 945
ceNPHP-4         GLDRFLTAQR----LDIQQRHEQLFNENSLDKIRQWNDLKEGFNFSDN-----KEIAQKF 858
                     :**  *     :  .*..:  ....*:::*. .  : * ::. .      . : :

hsNPHP4          QHLRDLQVIAAYRERTKAESIASLLSLAITTEHTLHATLGVAEFFEFVLKNPHNTQHTVT 1005
ceNPHP-4         IFEEELAAYKKLRYESKPAKLLEAVFKGITSCHQINPSFGEKVFFEFPLENYNSEPINCT 918
                  . .:* .    * .:*. .: . :  .**: * ::.::*   **** *:* :.   . *

hsNPHP4          VEIDNPELSVIVDSQEWRDFKGAAGLHTPVEEDMFHLRGSLAPQLYLRPHETAHVPFKFQ 1065
ceNPHP-4         IEFDDEALKPVFDAEEWKFYKTVNKVTTPSEKQMMRQT-TDRIEICLQPGDVLFIPFIYD 977
                 :*:*:  *. :.*::**: :* .  : ** *::*::   :   :: *:* :. .:** ::

hsNPHP4          SFSAGQLAMVQASPGLSNEKGMDAVSPWKSSAVPTKHAKVLFRASGGKPIAVLCLTVELQ 1125
ceNPHP-4         AFFFPNDAFNMYST----------------------KVVFRRWDTKEPLAILDLHVHRR 1014
                 :*   : *:   *.                        *: * .  :*:*:* * *. :

hsNPHP4          PHVVDQVFRFYHPELSFLKKAIRLPPWHTFPGAPVGMLGEDPPVHVRCSDPNVICETQNV 1185
ceNPHP-4         NFLLQHSVTFICETSGNWEKQLVLPP----------MARDRRVLSCRCSDPSVRLTVRNA 1064
                  .:::: . *     .  :* : ***          *  :   :  *****.*   .:*.

hsNPHP4          GPGEPRDIFLKVASGPSPEIKDFFVIIYSDRWLATPTQTWQVYLHSLQRVDVSCVAGQLT 1245
ceNPHP-4         T--LQQIVGFTTYSGETNDRKTFLLLMYSDHYQTRLMATWKITILPFFNVDVRSIVGQTT 1122
                      : : :.. ** : : * *::::***:: :    **:: : .: .*** .:.** *

hsNPHP4          RLSLVLRGTQTVRKVRAFTSHPQELKTDPKGVFVLPPRGVQDLHVGVRPLRAGSRFVHLN 1305
ceNPHP-4         RLHLLVHRRSEHDGVPDDLLKVYTASGCMKVVDSVLTERTPTATIDFTPNFIGTKKLVVS 1182
                 ** *:::  .    *     :    .   * *  : .. .    :.. *   *:: : :.

hsNPHP4          LVDVDCHQLVASWLVCLCCRQPLISKAFEIMLAAGEGKGVNKRITYTNPYPSRRTFHLHS 1365
ceNPHP-4         VVNTNTLKLERGFLVYGKSEAPRITQKFVIQIPSSD-EAIRKRIPIRNPYGLPKTFRITT 1241
                 :*:.:  :*  .:**   .. * *:: * * :.:.: :.:.***.  ***   :**:: :

hsNPHP4          DHPELLRFREDSFQVGGGETYTIGLQFAPSQRVGE-EEILIYINDHED-KNEEAFCVKVI 1423
ceNPHP-4         SNSDIVKITDSLLSVPPMGKLPCEMYFVKNTHLQKNIETLLYISDAETYVQEEAYSITLA 1301
                 .:.::::: :. :.*    . .  : *. . :: :  * *:**.* *   :***:.:.:

hsNPHP4          YQ-- 1425
ceNPHP-4         FEAS 1305
                 ::
```

Figure 2: Alignment of Homo sapiens MKS5 with Caenorhabiditis elegans mks-5

Figure 2 continued...

```
hsMKS5      IHINKVTFSSEVLQASGDKEPVT------FCTYAFYDFELQTTPVVRGLHPEYNFTSQYL 662
ceMKS5      INIPTPRVNTDLSVKLINVKPSPSLTSKFFFSLEFFDFQLETTPIMDAKQHNMDFTTVYD 838
            *:* .  ..:::     : :* .      * :  *:**:*:***:: . : : :**: *

hsMKS5      VHVNDLFLQYIQKNTITLEVHQAYSTEYETIAACQLKFHEILEK-SGRIFCTASLIGTKG 721
ceMKS5      VLVSNLLIHYLQTNGIVIEMYRPASDCYKLLAAATISLIPLFEDSVLRKFCSEIMLKSVD 898
            * *.:*:::*:*.* *.:*:::. *  *: :**. :.:  ::*.   * **:  :: : .

hsMKS5      DIPNFGTVEYWFRLRVPMDQAIRLYRERAKALGYITSNFKGPE----HMQSLSQQAPKTA 777
ceMKS5      TGVEMCTLRYEIEVSQPISDSFKKFKKSEMARNMLPLQLENEDTEDTNFDPLTIMVNRVV 958
               :: *:.* :.:  *:.:::: :::   * . :. :::. :    :::.*:  . :..

hsMKS5      QLSSTDSTDG----------NLNELHITIRCCNHLQSRASHLQPHPYVVYKFFDFADHDT 827
ceMKS5      GLDTFGKDPSTEFCIVDEFLSFSPYFTDFSTSSEIRSKRDCYIPKIDIARNLFATSSISF 1018
             *.: ..  .           .:.  .  :  ...::*: .   *:  :. ::*  :. .

hsMKS5      AIIP-SSNDPQFDDHMYFPVPMNMDLDRYLKSES--LSFYVFDDSDTQENIYIGKVNVPL 884
ceMKS5      FLIENIPRQDGVIATLHLPLHPLCKLGGSIKGTFPMLDTDGRPSSVSLDLCLIWKHEIPS 1078
             :*   ..:  .   :::*:    .*.  :*.    *.     .* : :   * * ::*

hsMKS5      ISLAHDRCISGIFELTDHQKHPAGTIHVILKWKFAYLPPSGSITTEDLGNFIRSEEP--E 942
ceMKS5      FFLKHEPKEP-LKEVKDTPILPQPVRRTSKEFVVTPVKEAELHDAEPTSMPPKAPEPTTA 1137
            : * *:   . : *:.*    *  . :.  :: .: : :   :    :*  .   :: **

hsMKS5      VVQRLPPASSVSTLVLAPRPKPRQRLTPVDKKVSFVDIMPHQSDETSPPLEDRKEISPEV 1002
ceMKS5      PLRRLSTDSSDTSFSHSSKDLFSPPTNPQTYDYEIPAVTPALVDSDGEEEADRIVFDDDD 1197
             ::**.. ** :::  :.:       .*   . .:  : *   *. .    **  :. :

hsMKS5      EHIPEIEINMLTVPHVPKVSQEGSVDEVKENTEKMQQG-----KDDVSLLSEGQLAEQSL 1057
ceMKS5      DEIESVSAVSSQRDPEPLEVPERQVENLPSPEDTPRPSDPLKPNGTNESKESTPVTQRSV 1257
            :.* .:.         *    * .*::: .  :. : .     :.  .  ..  ::::*:

hsMKS5      ASSEDETEITEDLEPEVEEDMS---ASDSDDCIIPGPISKNIKQSLALSPGLGCSSAISA 1114
ceMKS5      DKTDDVAPVDPELEPESGPEPEPVVESEPNEVAETEEDRKRELKTEELKSLLGALPPIAK 1317
             .::* : :  :****   : .    *:.::   .    *.  ::  *.. **. ..*:

hsMKS5      HCNFRL-PGSSDFPASASQVDGITG------ACHHSQPSEKIRIEIIALSLNDSQVTMDD 1167
ceMKS5      PRNIPVGPIALTEQPEATRQQGSTGRILFTDPLHFSVPPSESSSTSSPRRAEKAPVPLPD 1377
              *: : * :    ..*:: :* **       . *.* *..:      .   :.: *.: *

hsMKS5      TIQRLFVECRFYSLPAEETPVSLPKPKSGQWVYYNYSN-VIYVDKENNKAKRDILKAILQ 1226
ceMKS5      YEGHSLIKVRKPLSPTDKDVLEPNMKVSIQLETFELVPGSSLTPLTREETTFFVDWVFLD 1437
               : ::: *    *:::  :.     * *   ::       .   .:::.  :  .:*:

hsMKS5      KQEMPNRSLRFTVVSDPPEDEQDLECEDIGVAHVDLADMFQEGRDLIEQNIDVFDARADG 1286
ceMKS5      FTNEQSKSTIFDFPRRPQEMVDIRYTKEYTLTRGQLSLLDQWIRASIKFELTIIKISPGD 1497
              :  .:*  * .   * *  :    ::  ::: :*: : *  *  *: :: ::.  ...

hsMKS5      E---GIGKLRVTVEALHALQSVYKQYRDDLEA------------------- 1315
ceMKS5      EEELGFGSLILVPNNTQNKSFVIDVYDRSGIVQAEMTLTLHFSRALIEQLT 1548
            *   *:*.* :. :  :  . * . *  .  .
```

Dye-filling Defective Mutants

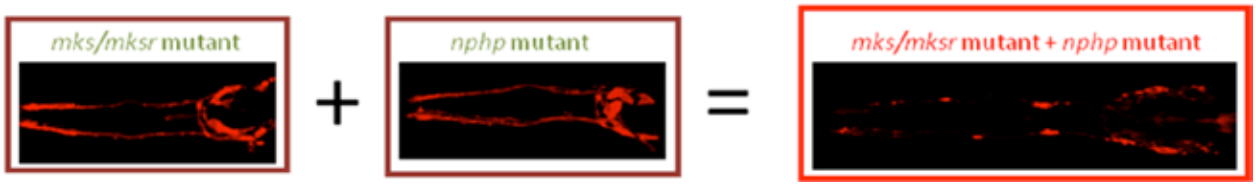

Figure 3 (above): mks mutant worms and nphp mutant worms individually dye fill normally. Only when there was a combination of an mks mutation along with an nphp mutation in the same worm did a dye-filling defect arise.

Figure 4 (below):Compilation of current mutant strains being studied. Strains 5, 39, and 91 were all isolated from this study and mapped to chromosome II. This showed that the induced mutation localized separately from the nphp-4(tm925) mutation along with inducing the Dyf phenotype.

Strain	Phenotype	Phenotype F1	Genotype Cross #1	Genotype Cross #2	Genotype Cross #3	Current Stage	Chromosome Location
yhw3	faint 1:15	wt	tm925	tm925	tm925	mapping	Chr IV
yhw 4	dyf		tm925	heterozygous		frozen	
yhw5	dyf	Wt	tm925	tm925	tm925	mapped	Chr II
yhw9	faint	Wt	tm925	tm925	tm925	mapping	
yhw10	faint					cross #1/ mating	
yhw12	very faint	Wt	tm925	tm925	tm925	mapping	
yhw14	dyf	Wt	tm925	tm925	tm925	cross #3 / genotyping	
yhw15	faint ~ 1:15	Wt	tm925	tm925	tm925	mapped	Chr V
yhw17	faint ~ 1:15	Wt	tm925	tm925	tm925	mapped	Chr V
yhw19	faint ~ 1:10	Wt	tm925	tm925	tm925	mapped	Chr V
yhw20	dyf	Wt	tm925	tm925	tm925	cross #3 / genotyping	
yhw22	dyf	Wt	tm925			cross #2 / genotyping	
yhw24	mix	Wt	tm925	tm925	tm925	cross #3 / genotyping	
yhw26	dyf	Wt	tm925	tm925	tm925	mapped	Chr X
yhw34	dyf	Wt	tm925	tm925	tm925	mapping	?
yhw35	dyf	Wt	tm925	tm925	tm925	mapping	
yhw36	faint ~ 1:15?*	Wt	tm925	tm925	tm925	mapping / outcross	
yhw39	dyf ~1:15?	Wt	tm925	tm925	tm925	mapped	Chr II
yhw40	dyf	Wt	tm925	tm925	tm925	mapping	
yhw62	dyf	Wt	tm925	tm925		cross #3 / genotyping	
yhw64	faint*	Wt	tm925	tm925	tm925	mapping	
yhw65	faint	Wt	tm925	tm925	tm925	mapped	Chr IV
yhw66	dyf	Wt	tm925	tm925	tm925	mapped	Chr IV
yhw 67			tm925	heterozygous		frozen	
yhw71	faint ~ 1:10	Wt	tm925	tm925	tm925	mapped	Chr X
yhw73	dyf	Wt	tm925			cross #2 / genotyping	
yhw91	very faint		tm925	tm925	tm925	mapped	Chr II
yhw103	dyf / roll					cross #1	
yhw 106	dyf		heterozygous				
yhw111	dyf	Wt	tm925	tm925		cross #3 / mating	
yhw112	dyf	Wt	tm925	tm925		cross #2 / genotyping	
Yhw 115	DYF		tm925	heterozygous		frozen	
yhw128	dyf < 1:15	Wt	tm925	tm925	tm925	mapped	Chr II
yhw129	dyf	Wt	tm925	tm925	tm925	mapping	
yhw130	dyf	Wt	tm925	tm925	tm925	mapping	
yhw131	dyf	Wt	tm925	tm925	tm925	mapping	
yhw146	dyf	Wt	tm925	tm925		cross #3 / mating	
yhw153	dyf	Wt	tm925			cross #2 / genotyping	
yhw165	very faint	Wt	tm925	tm925	tm925	mapped	Chr IV
yhw166	dyf	Wt	tm925	tm925	tm925	mapping	

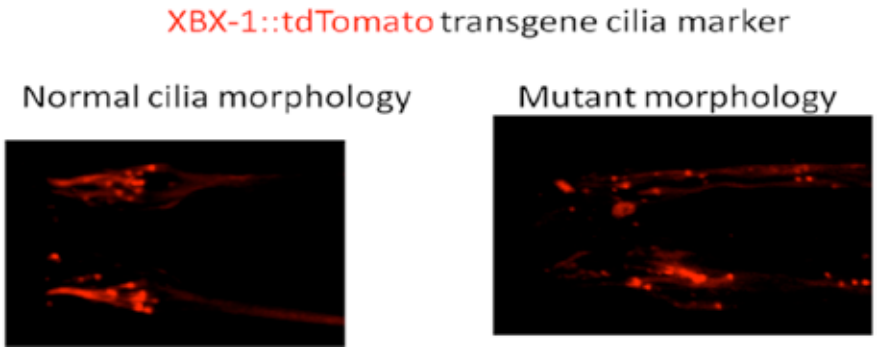

Figure 5: Morphology of the wild-type and experimental mutant strain expressing XBX-1::tdTomato cilia imaging marker. The mutant cilia morphology is noticeably misaligned. This accounts for the Dyf phenotype.

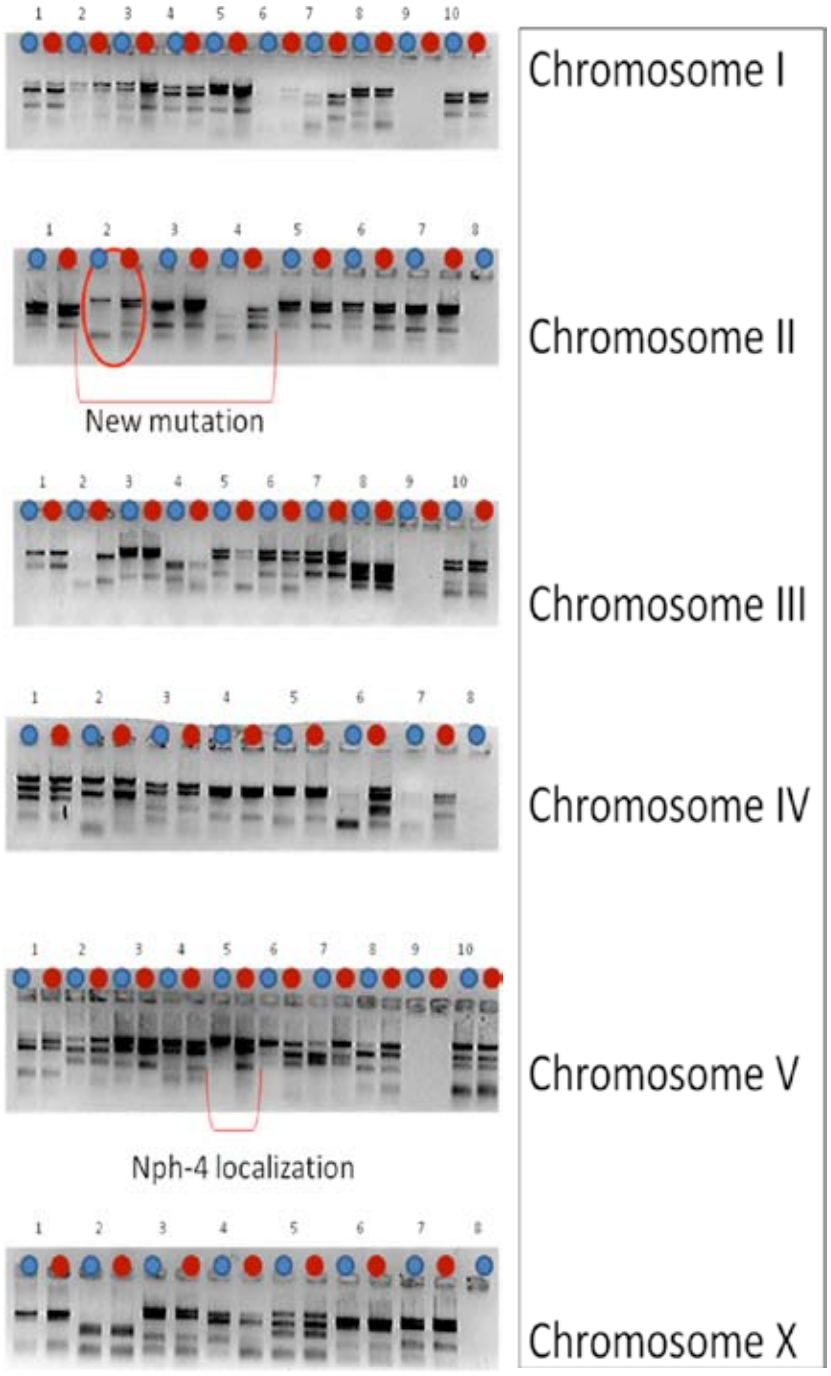

Figure 6: SNP mapping of strain 39.3. Visible mutations occurred on Chromosome II and V. nphp-4(tm925) localizes to chromosome V and the induced mutation localized to Chromosome II.

Dye-filling in NPHP/MKS compound mutant *C. elegans*

	mks-1	*mks-3*	*mks-5*	*mks-6*	*nphp-4*
mks-1	WT	WT	ND	ND	Dyf
mks-3	WT	WT	ND	ND	Dyf
mks-5	ND	ND	WT	ND	Dyf
mks-6	ND	ND	ND	WT	Dyf
nphp-4	Dyf	Dyf	Dyf	Dyf	WT

Table 1: Of the compound mutations studied solely between mks genes, there was a consistent wild-type phenotype. Induced compound mutations in an mks gene in combination with the nphp-4(tm925) resulted in a Dye-filling defective phenotype (Dyf).

analysis to uncover the molecular identity of the mutations. After sequencing, transgenic rescue experiments will be performed to confirm the identified mutant gene is responsible for the Dyf phenotype. Following this confirmation, the localization of the corresponding protein and its role in regulating cilia assembly can be explored. Further studies will involve screening for mutations in this gene in human patients with cilia-related disorders. [3,5]

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