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ORIGINAL ARTICLE

Analysis of dynein intermediate chains, light intermediate chains and light chains in a cohort of hereditary peripheral neuropathies

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Abstract The cytoplasmic dynein heavy chain (*DYNC1H1*) gene has been increasingly associated with neurodegenerative disorders including axonal Charcot–Marie–Tooth disease (CMT2), intellectual disability and malformations of cortical development. In addition, evidence from mouse models (*Loa*, catabolite repressor–activator (*Cra*) and Sprawling (*Swl*)) has shown that mutations in *Dync1h1* cause a range of neurodegenerative phenotypes with motor and sensory neuron involvement. In this current study, we examined the possible contribution of other cytoplasmic dynein subunits that bind to *DYNC1H1* as a cause of inherited peripheral neuropathy. We focused on screening the cytoplasmic dynein intermediate, light intermediate and light chain genes in a cohort of families with inherited peripheral neuropathies. Nine genes were screened and ten variants were detected, but none was identified as pathogenic, indicating that cytoplasmic dynein intermediate, light intermediate and light chains are not a cause of neuropathy in our cohort.

Keywords Dynein · Dynein intermediate chains · Dynein light intermediate chains · Dynein light chains · Charcot–Marie–Tooth (CMT) · Hereditary sensory neuropathies (HSN)

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Introduction

Inherited peripheral neuropathies (IPN) are a large group of disorders that can manifest in motor and/or sensory phenotypes and are increasingly recognised for their phenotypic and genetic heterogeneity. Over 40 genes have been implicated in these disorders, and despite the wide diversity of function, the phenotypes that result from these mutated genes commonly share a progressive degeneration of the peripheral nerves.

The cytoplasmic dynein–dynactin complex plays an important role in microtubule dynamics and in the transport of cargo and organelles within cells. In neurons, cytoplasmic dynein is responsible for the retrograde transport along axons and also plays a role in neuronal migration [14, 37]. Cytoplasmic dynein is a large protein complex comprising of many different subunits including the heavy chain, intermediate chains, light intermediate chains and the light chains (Fig. 1). These subunits are encoded by ten genes and include the heavy chain (*DYNC1H1*), intermediate chains 1 and 2 (cytoplasmic dynein intermediate chain 1 (*DYNC1I1*) and cytoplasmic dynein intermediate chain 2 (*DYNC1I2*)), light chains (cytoplasmic dynein light chain (*DYNLL*) *1–L2*, cytoplasmic dynein light chain Roadblock 1 (*DYNLRB1*), cytoplasmic dynein light chain Tctex (*DYNLT*) *1* and *DYNLT3*) and light intermediate chains (cytoplasmic dynein light intermediate chain 1 (*DYNC1LI1*) and cytoplasmic dynein light intermediate chain 2 (*DYNC1LI2*)). This complex then further associates with the dynactin complex which is comprised of subunits encoded by eight genes (dynactin (*DCTN*) *1–6*, *ACTR1A* and *ACTR1B*). The subunits within the cytoplasmic dynein–dynactin complex play a role in specifying which cargo is transported along the axon, namely signalling and trophic factors. Mouse models with mutations in cytoplasmic dynein–dynactin genes are known to exhibit a range of neurodegenerative phenotypes with motor and sensory involvement [4, 12, 21] (Fig. 1). Mice bearing *Dync1h1* mutations

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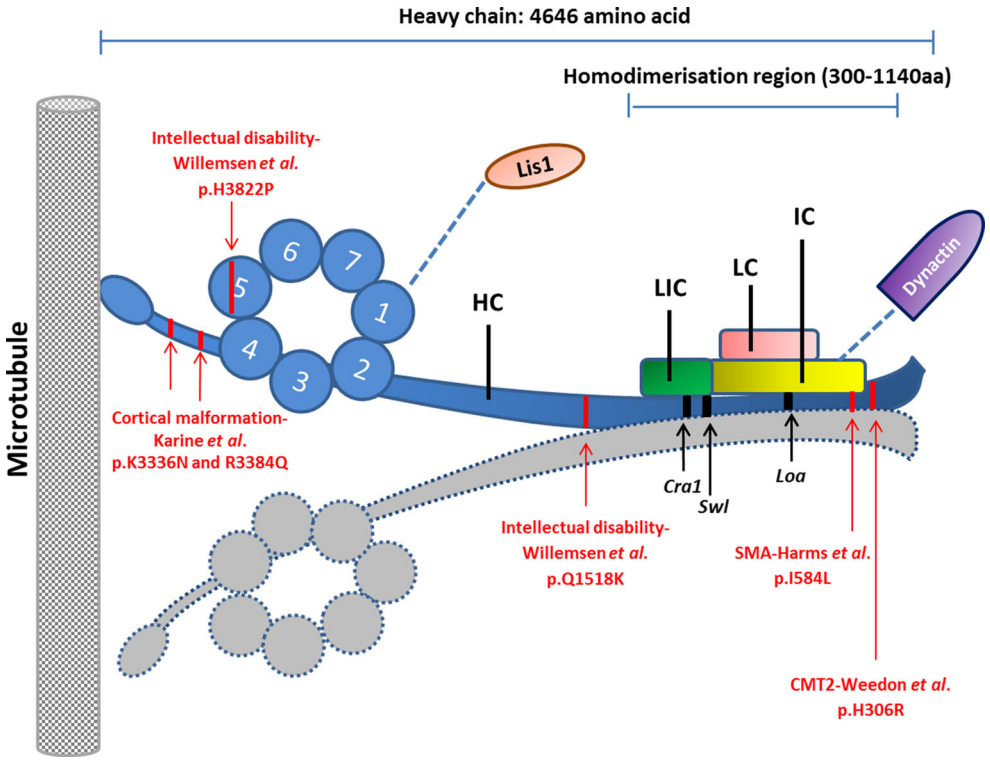

Fig. 1 A schematic diagram of the cytoplasmic dynein heavy chain and the dynein intermediate, light intermediate and light chain subunits and their location within the dynein–dynactin complex. Known mutations reported in mice and humans are indicated. Individual dynactin subunits are not shown in this figure. *HC* heavy chain, *IC* intermediate chain, *LIC* light intermediate chain, *LC* light chain

(*Swl*, *Cra1* and *Loa* mice) demonstrated an early-onset proprioceptive sensory neuropathy which correlated with the loss of proprioceptive DRG neurons and muscle spindle degeneration [4, 10]. More recently, mutations in DYNC1H1 were reported in an axonal form of Charcot-Marie-Tooth (CMT) disease and spinal muscular atrophy, and interestingly, two other groups found that mutations in the same gene were responsible for a type of intellectual disability and malformations of cortical development [13, 19, 43, 44] (Fig.1).In addition, *DCTN1* has been associated with a form of human spinal bulbar muscular atrophy and amyotrophic lateral sclerosis [27, 32, 33], and an isoform of an anterograde motor protein, kinesin (*KIF1B*), has also been implicated in CMT type 2A [46], although this appears to be a rare cause of CMT2 as this finding has not been replicated in other cases across the world. A number of reports have recently described mutations in the bicaudal D homolog 2 (*BICD2*) gene in spinal muscular atrophy (SMA) or hereditary motor neuropathy (HMN) and hereditary spastic paraplegia (HSP) [29, 28 30]. The *BICD2* gene is a key protein involved in numerous cellular activities relevant to dynein–dynactin–kinesin-mediated transport [25, 15, 38] and in organelle localisation within the cell [8]. The involvement of these genes provides evidence suggesting that perturbed axonal transport can lead to neurodegenerative phenotypes.

In this study, we examined the possible contribution of genes within the cytoplasmic dynein complex in a cohort of families with IPNs including hereditary sensory neuropathy (HSN), CMT and HMN. Whilst members of the dynein family are known to cause CMT, no studies to date have investigated if mutations in cytoplasmic dynein genes are a cause for HSN or HMN.

To address the hypothesis that these genes play a role in IPNs, we screened the coding exons of the intermediate chains, light intermediate chains and light chains using a combination of high-resolution melt (HRM) analysis and whole exome sequencing (WES) to identify variants in a cohort of IPN families. In total, we found ten variants in our cohort (nine reported in the Single Nucleotide Polymorphism Database (dbSNP) and one reported in the NIH National Heart, Lung, and Blood Institute (NHLBI) database). However, none of the variants is pathogenic.

Methodology

Patient selection

One hundred and thirty-six families were recruited through the Neurogenetics Clinic Concord Hospital, Sydney, and diagnosed with IPNs based on clinical features consistent with a genetic neuropathy. The classes of IPNs and the number of patients are listed in Table 1. The CMTX and HMNX families are most likely X-linked as they showed no male to male transmission, and females had a less severe phenotype. However, due to the small size of the pedigrees, the possibility

Table 1 Amongst the 136 families, 76 are diagnosed with CMT, 29 with HSN and 31 with HMN

Classes of IPNs	Number of patients screened	WES	HRM
CMT (n=76)			
CMT1	4	4	0
CMT2	43	43	0
CMT4	1	0	1
CMT5	3	2	1
CMTX	25	25	0
HSN (n=29)			
HSN1	28	1	27
HSN2	1	0	1
Other (n=31)			
HMN	14	14	0
HMNP	15	15	0
HMNX	2	2	0
Total	136	106	30

CMT Charcot–Marie–Tooth disease, *HSN* hereditary sensory neuropathy, *HMN* hereditary motor neuropathy, *X* X-linked query, *HMNP* HMN with pyramidal signs

of autosomal dominant inheritance could not be entirely excluded. Prior to this study, as part of routine diagnostic procedures, families were screened for common genes (*PMP22*, *MPZ*, *MFN2*, *GJB1*and *SPTLC1*) based on clinical presentation, and they were all negative.

Ethics

Patient ascertainment and collection of blood samples for DNA analysis was performed with informed consent according to protocols approved by the Sydney Local Health District, Human Ethics Committee, Concord Hospital, Australia.

Mutation screening

Nine genes comprising the cytoplasmic dynein intermediate chains (*DYNC1I1* and *DYNC1I2*), cytoplasmic dynein light intermediate chains (*DYNC1LI1* and *DYNC1LI2*) and cytoplasmic dynein light chains (*DYNLL1*, *DYNLL2*, *DYNLT1*, *DYNLT3* and *DYNLRB1*) underwent mutation screening. Genomic DNA was isolated from whole blood using the Puregene kit (Qiagen) according to the manufacturer's recommendations. Mutation screening was performed either by HRM analysis or WES.

HRM analysis

PCR amplicons for mutation scanning were designed to include the coding exons and flanking intronic sequences using the LightScanner primer design software 1.0 (Idaho Technology Inc.). The gene reference sequences are shown in Table 2. Each amplicon was amplified using HRM Master Mix (Idaho Technology) as previously described [20]. PCR conditions and primer sequences are available upon request.

Melt acquisition was performed on a 96-well LightScanner (Idaho Technology Inc.) with melt temperatures ranging from 62 to 98 °C, and the data were analysed using the LightScanner Software Call-IT 2.0. Amplicons with differential melt curves were sequenced using BigDye Terminator Cycle Sequencing protocols at the Australian Cancer Research Foundation (ACRF) Facility, Garvan Institute of Medical Research, Australia. The sequence data were aligned to the Human Genome February 2009 (GRCh37/hg19) assembly to identify possible variants.

WES analysis

Whole exome sequencing of DNA from index family members was carried out using the exome sequencing service from Axeq Technologies (South Korea). In brief, sequencing libraries were prepared using the Ilumina TruSeq kit and sequenced as 100-bp paired end reads on the HiSeq 2000 Sequencer (Illumina, San Diego, California). Sequence reads were aligned to the Human Genome February 2009 (GRCh37/hg19) assembly using BWA software [22]. Duplicate reads were removed using Picard software (http://picard.sourceforge.net/). Variants (single nucleotide polymorphisms and indels) were called using SAMtools [23] and annotated with dbSNP, the 1,000 Genomes SNP call release (20101109) and NHLBI ESP databases using ANNOVAR [41]. All but one of the 106 samples achieved a mean read depth of >30× for the target regions (range 27.5×–88×). This achieved ≥90 % sensitivity to detect heterozygous variants within the exome target regions [5]. Variants identified with WES were confirmed using Sanger sequencing of PCR amplicons at the ACRF sequencing service facility (Garvan Institute, Australia).

Segregation analysis

Sequence variants with a minor allele frequency (MAF) of less than 1 % were genotyped in additional family members using Sanger sequencing and assessed for segregation with the phenotype in the pedigree.

Results

We screened a total of 136 cases (76 CMT, 29 HSN and 31 HMN) for mutations in nine genes within the cytoplasmic

Table 2 Gene IDs for the cytoplasmic dynein intermediate, light intermediate and light chain genes

Name	Symbol	Chromosome position	Gene ID	No. of exons
Cytoplasmic dynein intermediate chain 1	*DYNC1I1*	7q21.3-22.1	NM_004411	17
Cytoplasmic dynein intermediate chain 2	*DYNC1I2*	2q31.1	NM_001378.1	18
Cytoplasmic dynein light intermediate chain 1	*DYNC1LI1*	3p22.3	NM_016141.3	13
Cytoplasmic dynein light intermediate chain 2	*DYNC1LI2*	16q22.1	NM_006141.2	13
Cytoplasmic dynein light chain 1 (LC8 type 1)	*DYNLL1*	12q24.23	NM_003746	3
Cytoplasmic dynein light chain 2 (LC8 type 2)	*DYNLL2*	17q22	NM_080677.2	3
Cytoplasmic dynein light chain Roadblock 1	*DYNLRB1*	20q11.21	NM_014183	4
Cytoplasmic dynein light chain Tctex 1	*DYNLT1*	6q25.2	NM_006519.2	5
Cytoplasmic dynein light chain Tctex 3	*DYNLT3*	Xp21	NM_006520	6

dynein complex (Table 1). A total of ten variants were detected in 43 patients (Table 3).

Cytoplasmic dynein intermediate chains

Through WES and HRM screening, a total of four *DYNC1I1* variants were identified (Table 3). Two synonymous variants were detected (rs3757697 and rs1048666). The rs3757697 variant was detected in the CMT, HSN and HMN cohort, and rs1048666 was detected in the HSN cohort (Table 3). As these variants do not change the encoded amino acids and have high minor allele frequencies of 10.2 % (rs3757697) and 24.5 % (rs1048666), they are unlikely to be pathogenic.

We detected two non-synonymous variants in CMT5 and CMTX patients, rs35314029 (MAF 1.3 %) and TMP_ESP_7_95499296 (MAF 0.01 %), respectively (Table 3). These variants did not segregate with the disease in the respective families and are therefore unlikely to be pathogenic. No variants were found in *DYNC1I2* by either WES or HRM screening.

Cytoplasmic dynein light intermediate chains

Four non-synonymous variants were detected in *DYNC1LI1* (Table 3). The rs2303857 variant is a common polymorphism with a MAF of 6.5 % and was detected in the CMT, HSN

Table 3 Variants detected either by WES or HRM

Gene	SNP ID	Amino acid change	Segregation/Validation results	MAF (dbSNP)	No. of individuals with the variant in CMT families	No. of individuals with the variant in HSN families	No. of individuals with the variant in HMN families
DYNC1I1	rs3757697	p.Q155Q	Synonymous variant	10.2 %	1/76	5/30	1/31
	rs1048666	p.G497G	Synonymous variant	24.5 %		6/30	–
	rs35314029	p.L199M	No segregation with phenotype	1.3 %	1/76	–	–
	TMP_ESP_7_95499296	p.T176M	No segregation with phenotype	0.01 % (NHLBI)	1/76	–	–
DYNC1LI1	rs143775988	p.G77R	Segregation analysis not performed as DNA from additional family members was not available	0.1 %	–	1/30	–
	rs138677120	p.V139I	No segregation with phenotype	1.0 %	4/76	3/30	1/31
	rs116075738	p.V146A	No segregation with phenotype	0.1 %	–	–	1/31
	rs2303857	p.Q277R	Common polymorphism	6.5 %	12/76	4/30	5/31
DYNC1LI2	rs10990	p.V412V	Synonymous variant	1.1 %	–	2/30	–
DYNLT3	rs11771	p.A61A	Synonymous variant	30.4 %	1/76	–	–

All MAF values were obtained from dbSNP137 unless stated otherwise

NHLBI NIH National Heart, Lung and Blood Institute database, *MAF* minor allele frequency

and HMN cohorts. Other three variants (rs138677120, rs116075738 and rs143775988) have MAFs of less than 1.0 %. The rs138677120 variant was detected in two CMT2 patients, two CMTX patients, three HSN1 patients and one HMNP patient, whilst rs116075738 was detected in one HMN patient (Table 3). The rs138677120 and rs116075738 variants did not segregate with the disease in the respective families (Table 3). The rs143775988 variant was detected in an HSN1 patient and has a reported MAF of 0.1 %. Segregation analysis was not possible as DNA from additional family members was not available (Table 3).

The synonymous rs10990 variant was identified in *DYNC1LI2* in two HSN1 patients, and although it has a low MAF of 1.1 %, it is unlikely to be pathogenic as the encoded amino acid is unchanged (Table 3).

Cytoplasmic dynein light chains

We examined members of the cytoplasmic dynein light chain family (*DYNLL1*, *DYNLL2*, *DYNLRB1*, *DYNLT1* and *DYNLT3*). Only one variant was identified through WES and HRM analysis in this family of genes (Table 3), which was a synonymous variant, rs11771 with a MAF of 30.4 %, found in *DYNLT3* in a patient with CMTX (Table 3). As this variant did not change the encoded amino acid, it is likely to be non-pathogenic.

Discussion

In this study, we used a candidate gene approach to identify possible mutations in the cytoplasmic dynein complex in patients with IPN. Using a combination of HRM mutation screening and WES, we found four variants in both the *DYNC1I1* and *DYNC1LI1* genes and a single variant in each of the *DYNC1LI2* and *DYNLT3* genes.

Dynein intermediate chains

The dynein intermediate chain is encoded by two genes, *DYNC1I1* and *DYNC1I2*. There are currently no reported disease-causing mutations in these two genes although several phenotypes have been described in *Drosophila* and *Dictyostelium*. In *Dictyostelium*, overexpression of the intermediate chain causes disruption to the Golgi complex and defects in microtubule organisation and cell division [3, 11, 24], and in *Drosophila*, mutations in these genes result in larval lethality and a crawling phenotype, which has been attributed to axonal transport abnormalities (Boylan et al. 2002). A possible link between CMT and dynein intermediate chains may be identified through studies on Rab7-associated CMT, whereby *RAB7* mutations can cause CMT type 2B [40], and transport of *RAB7*-positive endosomes in cultured neurons is disrupted if intermediate dynein chains are abnormally phosphorylated [26].

For *DYNC1I1*, the non-synonymous variant (rs35314029) resides in the postulated *DYNLT* (Tctex dynein light chain) and *DYNLL* (LC8 dynein light chain) binding domains, while the TMP_ESP_7_95499296 variant is in the dynein intermediate chain dimerisation domain. Segregation analysis has excluded these non-synonymous *DYNC1I1* variants as being pathogenic in our IPN cohort.

Although no pathogenic variants were found, these genes remain interesting candidates for further investigation as they play an integral role by binding to the dynein heavy chain and acting as the 'adaptor' for other subunits. Interestingly, the mutation in *DYNC1H1* which causes axonal CMT [43] is located in the homodimerisation/binding site for the intermediate chain genes and may play a role in the development of the disease by disturbing the binding of specific intermediate chain subunits and their associated cargos to the dynein complex.

Dynein light intermediate chains

The light intermediate chains are encoded by two genes, *DYNC1LI1* and *DYNC1LI2*. These genes are thought to bind to specific cargo (for example, pericentrin—[9, 34, 39]) and help maintain the stability of the cytoplasmic dynein–dynactin complex. Recently, a point mutation in *Dyncili1* was reported to result in anxiety-like behaviour and cause defects in nervous system development in mice [1]. Mutations in the *Drosophila* dynein light intermediate chain genes have been reported to give rise to defects in dendritic and axonal morphology [36, 47]. Similar to the dynein intermediate chains, the light intermediate chains can also bind to the Rab protein family, in particular *Rab11* [16], *Rab5* [36], *Rab6* [42] and *Rab4a* [2]. *Rab4A* interacts with *NDRG1*, in which mutations can cause hereditary motor and sensory neuropathy Lom (HMSNL/CMT4D) [17, 18]. This network of interconnected pathways involving dyneins and *Rab* genes is an interesting avenue for further research into IPNs.

Four variants were detected in *DYNC1LI1* and one variant in *DYNC1LI2*. These variants are likely to be non-pathogenic. Although the *Dync1li1* mice did not show any defects in the developing peripheral nerves [1], it is possible that the mutation may give rise to late-onset peripheral neuropathy which was not examined in these mice.

Dynein light chains

Cytoplasmic dynein light chains are composed of three families: *DYNLL* (LC8 family), *DYNLT* (T-complex family) and *DYNLRB* (Roadblock family). Only one variant, a synonymous SNP, (rs11771 in *DYNLT3*) was detected in the cytoplasmic dynein light chain genes. In *Drosophila*, mutations in

the *DYNLL* (LC8) gene disrupt the sensory neuron projections, and neurite outgrowth is disrupted in mice with *Dynlt1* mutations [6, 31, 35].

Our study has focused on IPNs, although the impact of different mutations in these genes could result in other neurological diseases, by affecting the binding of specific subunits. Recent studies by Hafezparast and colleagues indicate that *Dync1h1* mutations can perturb the binding affinities of the dynein intermediate chains, light intermediate chains and light chains (specifically *Dynlt1*) and compromise the interaction between dynactin and the dynein intermediate chains [7]. Similarly, any mutations within the heavy chain dimerisation sites of the dynein intermediate, light intermediate and light chain genes could potentially have the same functional effect, as the cytoplasmic dynein complex would be uncoupled from the dynactin complex, leading to the loss of transport of essential factors.

Conclusion

No pathogenic mutations were found in the nine genes screened, *DYNC1I1*, *DYNC1I2*, *DYNC1LI1*, *DYNC1LI2*, *DYNLL1*, *DYNLL2*, *DYNLT1*, *DYNLT3* and *DYNLRB1*, in an IPN cohort using a combination of targeted mutation screening by HRM analysis and WES. Nevertheless, in view of the important role that they play in the cytoplasmic dynein–dynactin complex, these genes are attractive candidates for neurological disorders and warrant further investigation.

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