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RESEARCH ARTICLE

KIF5A and KLC1 expression in Alzheimer's disease: relationship and genetic influences [version 1; peer review: 1 approved, 1 approved with reservations, 1 not approved]

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Abstract

Background: Early disturbances in axonal transport, before the onset of gross neuropathology, occur in a spectrum of neurodegenerative diseases including Alzheimer's disease. Kinesin superfamily motor proteins (KIFs) are responsible for anterograde protein transport within the axon of various cellular cargoes, including synaptic and structural proteins. Dysregulated KIF expression has been associated with AD pathology and genetic polymorphisms within kinesin-light chain-1 (KLC1) have been linked to AD susceptibility. We examined the expression of KLC1 in AD, in relation to that of the KLC1 motor complex (KIF5A) and to susceptibility genotypes.

Methods: We analysed KLC1 and KIF5A gene and protein expression in midfrontal cortex from 47 AD and 39 control brains.

Results: We found that gene expression of both *KIF5A* and *KLC1* increased with Braak tangle stage (0-II vs III-IV and V-VI) but was not associated with significant change at the protein level. We found no effect of KLC1 SNPs on KIF5A or KLC1 expression but KIF5A SNPs that had previously been linked to susceptibility in multiple sclerosis were associated with reduced *KIF5A* mRNA expression in AD cortex.

Conclusions: The findings raise the possibility that genetic polymorphisms within the *KIF5A* gene locus could contribute to disturbances of axonal transport, neuronal connectivity and function across a spectrum of neurological conditions, including AD.

Keywords

Alzheimer's disease, axonal transport, KIF5A, kinesin light chain-1, single nucleotide polymorphism

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Introduction

Over 90% of Alzheimer's disease (AD) cases are sporadic rather than attributable to a single gene mutation. In these cases, as for many other sporadic adult-onset neurodegenerative diseases, the pathological changes are likely to be caused by a combination of environmental and genetic factors that activate variably independent pathogenic pathways that increase neuronal vulnerability to damage and death[1]. One major genetic risk factor for sporadic disease is the ε4 allele of *APOE*[2]. Aside from *APOE*, genome-wide association studies (GWAS), have linked single nucleotide polymorphisms (SNPs) in over 20 genetic loci to AD susceptibility[3].

A common theme across neurodegenerative diseases is alterations in neuronal connectivity that can give rise to clinical symptoms before neuronal loss[4,5]. Targeting the processes responsible for the changes in neuronal connectivity is a key therapeutic aim, before consequential axonal loss and neuronal death cause irreversible disability in patients with neurodegenerative disease[6,7]. A crucial step in this transition from potentially reversible to irreversible disease is the disruption in axonal delivery of membrane-bound organelles from the neuronal soma to the synapse. The development of such disruption has been recognised pathologically in brain tissue, including in AD[8], by the formation of axonal swellings at an early stage in the disease process.

Kinesin superfamily motor proteins (KIFs) are responsible for most anterograde protein transport within the axon of various cellular cargoes, including synaptic and structural proteins[9]. There are at least 38 different neuronally-enriched KIFs which contain a conserved microtubule-binding domain and a motor domain at their amino (NH_2-) terminus, which hydrolyses ATP in order to generate motile forces to shift associated cargoes along the axon, via microtubule tracks[10]. The heterogeneous tail (COOH-terminal) domain of KIFs determines their cargo-binding specificity[11].

Early disturbances in axonal transport are a feature of several neurodegenerative diseases[12–14], and studies have linked KIF dysregulation to AD[15,16]. Conventional kinesin member KIF5A transports several cargoes, including APP and β- and γ- secretases, by complexing with kinesin light chain-1 (KLC1)[17,18]. Deletion of the KLC1 subunit in mice leads to early selective axonal transport defects, resulting in a disorganised neuronal cytoskeleton with APP, neurofilament (NF) and hyperphosphorylated tau accumulation[19]. Mice transgenic for human APP with mutations that cause familial AD develop abnormal axonal swellings positive for KLC1 and structural axonal component phosphorylated NF-H, long before AD pathology can be detected[8,20].

Single nucleotide polymorphisms (SNPs) in the region of the *KLC1* gene have been linked to AD susceptibility; in particular, *rs8702* has been shown to predict conversion of mild cognitive impairment (MCI) to AD[16,21,22]. Our previous studies have shown that SNPs within the *KIF5A* gene locus, linked to multiple sclerosis (MS) susceptibility, influence KIF5A expression in post-mortem brain[23,24].

We and others have found KIF5A expression to be increased in post-mortem AD and PD tissue[25,26]. KIF members show a degree of functional redundancy, such that more than one KIF may transport a specific cargo[27]. We therefore suggested that KIF5A might be upregulated in AD as compensatory protective response to defects in other, interoperable, axonal transport proteins, facilitating the transport and clearance of aggregating cargoes in the cell body and axon. In support of this hypothesis, we showed that KIF5A levels in both MS and AD correlated inversely with the amount of cargo APP[24,25]. KIF5A shares many cargoes with anterograde motor protein KIF1B, such as mitochondria and synaptic vesicle proteins, and it can transport cargoes without complexing to KLC1[9,28].

In this study we aimed to determine whether KLC1 levels are altered in AD, the relationship between the levels of KLC1 and KIF5A, and whether expression of KLC1 in the cerebral cortex is influenced by *KLC1* polymorphisms, which have been linked to AD susceptibility.

Methods

Study cohort

Samples of midfrontal cortex (Brodmann area 8; 500mg) were obtained from the South West Dementia Brain Bank (Bristol, UK), under the terms of South West - Central Bristol Research Ethics Committee approval no 08/H0106/28+5. Our previous studies indicated an influence of post-mortem delay (PMD) on mRNA and protein expression in brain tissue[25]. Therefore, cases with a PMD >72 h were excluded from analysis. We studied 47 cases of AD (in which, AD neuropathological change was an adequate explanation of dementia, according to National Institute on Aging-Alzheimer's Association guidelines[29]), and 39 age-matched controls with no history of cognitive impairment (Table 1).

RNA and protein extraction

Both RNA and protein were extracted from each sample by use of the Paris™ Kit (ThermoFisher Scientific; AM1921), according to the manufacturer's instructions. Samples were weighed, divided into 2x 50 mg portions and placed in 2 ml homogenisation tubes, pre-filled with 5x 2.3 mm silica beads (Stratech Scientific; 11079125z-BSP) and 300 µL of Paris™ kit lysis buffer, with the addition of Halt™ Protease and phosphatase inhibitor cocktail and 0.5M EDTA (1:100) (ThermoFisher Scientific; 78440). Samples were homogenised using a Precellys®24 automated homogeniser (Stretton Scientific). Samples were subsequently centrifuged (Sorvall ST 16R) at 10,000 xg for 2 min at 4°C and the soluble protein supernatant removed and stored at -80°C until required. The remaining homogenate was taken through the additional manufacturer's steps for extraction and elution of RNA, before storage at -80°C.

gDNA extraction

An approximately, 2 mm slice was taken from each brain sample and gDNA extracted using the TaqMan® Sample-to-SNP™ Kit (ThermoFisher Scientific; 4403313). Cortex was placed in lysis buffer, briefly centrifuged and incubated for 3 min at 95°C, before addition of DNA stabilising solution. Samples were then stored at -20°C until use.

Table 1. Clinical characteristics of Alzheimer's disease and control patient cohort.

Patient ID	Age (yrs)	Sex (M/F)	Post-mortem delay (hrs)	Braak stage
C 1	62	M	4	0
C 2	95	F	46	0
C 3	78	F	24	2
C 4	64	M	12	2
C 5	64	M	16	0
C 6	90	M	45	2
C 7	64	M	23	2
C 8	77	M	55	1
C 9	78	M	12	2
C 10	73	M	36	2
C 11	88	F	62	2
C 12	88	F	72	0
C 13	93	F	18	2
C 14	88	F	28	2
C 15	82	M	30	2
C 16	84	M	48	3
C 17	90	M	48	2
C 18	75	M	48	2
C 19	89	F	15	2
C 20	73	M	33	1
C 21	69	M	66	2
C 22	73	F	59	1
C 23	83	F	24	2
C 24	82	M	3	2
C 25	79	M	24	-
C 26	43	F	12	-
C 27	84	F	17	1
C 28	82	F	37	2
C 29	78	M	48	1
C 30	82	M	56	2
C 31	76	M	23	2
C 32	91	F	60	2
C 33	77	M	10	3
C 34	75	M	6	3
C 35	78	M	21	-
C 36	93	F	53	3
C 37	90	M	5.5	2
C 38	90	F	67.25	1
C 39	83	M	47.75	2
Mean	**80 (+/- 11)**	**-**	**34 (+/- 20)**	**-**
AD 1	89	F	71	5
AD 2	78	F	9	5
AD 3	81	F	42	6
AD 4	91	F	37	4
AD 5	77	F	43	4
AD 6	96	F	53	4
AD 7	87	F	67	5
AD 8	79	F	70	3
AD 9	81	M	29	4
AD 10	91	F	70	5
AD 11	78	F	35	6
AD 12	83	F	43	5
AD 13	70	F	25	6
AD 14	78	F	4	5
AD 15	69	M	48	5
AD 16	74	M	50	5
AD 17	80	F	21	5
AD 18	95	M	48	3
AD 19	89	F	4	6
AD 20	79	M	28	6
AD 21	85	M	66	6
AD 22	81	F	66	4
AD 23	80	M	31	6
AD 24	90	F	21	4
AD 25	57	F	24	5
AD 26	54	F	24	6
AD 27	84	F	20	5
AD 28	93	M	20	3
AD 29	80	M	5	6
AD 30	87	F	55	6
AD 31	74	M	24	5
AD 32	78	F	21	5
AD 33	89	F	39	5
AD 34	84	M	64	5
AD 35	73	F	38	5
AD 36	68	M	61	6
AD 37	83	M	48	5
AD 38	74	M	48	5
AD 39	78	M	49	6
AD 40	78	M	50	6
AD 41	85	M	50	6
AD 42	98	F	21	5
AD 43	83	F	32	6
AD 44	69	M	12	5
AD 45	87	F	28	6
AD 46	84	F	21	6
AD 47	84	F	22	5
Mean	**81 (+/- 9)**	**-**	**37 (+/- 19)**	**-**

Abbreviations: AD: Alzheimer's disease; C: control; F: female; hrs: hours; M: male; yrs: years.

Complementary DNA (cDNA) generation

Extracted RNA was quantified using the Qubit® RNA HS Assay Kit (ThermoFisher Scientific; Q32852) and Qubit® 4.0 fluorometer (excitation 600-645 nm and emission 665-720 nm) (ThermoFisher Scientific; Q33226), according to the manufacturer's instructions. Quantified RNA samples were subsequently treated with recombinant RNase-free DNase I (10,000U; Sigma Aldrich; 4716728001) and 50 mM $MgCl_2$ solution (Bioline; BIO-37026) to remove any trace DNA. Treated RNA was diluted to achieve 100 ng/50 µL for reverse transcription into cDNA using the High Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific; 4368814), according to the manufacturer's instructions. 50 µL of reverse transcription mastermix was added to diluted RNA samples before thermal cycling on a StepOnePlus PCR system™ for cDNA generation. The cycling conditions were holding at 25°C for 10 min, 37°C for 120 min, followed by 85°C for 5 min. cDNA was stored at -80°C until use.

Quantitative real-time PCR (qPCR)

cDNA (2 ng/µL) was diluted to a final volume of 20 µL in TaqMan® 2x Fast Advanced Master Mix (ThermoFisher Scientific; 4444557) and TaqMan® gene expression probe solution (FAM-MGB dye-labelled, ThermoFisher Scientific) for *KIF5A* (Hs01007893_m1), *KLC1* (Hs00194316_m1) *RBFOX3* (Hs01370653_m1) and *GAPDH* (Hs02758991_g1). qPCR was performed on a StepOnePlus™ Real-Time PCR system with StepOne software v2.1 (ThermoFisher Scientific), on a FAST ramp speed, (Holding at 50°C for 2 min, 95°C for 2 min, cycling (x40) at 95°C for 1 s, followed by 60°C for 20 s). As the genes of interest are expressed predominantly neuronally, gene expression was calculated relative to that of a neuron-specific calibrator gene, encoding neuronal nuclear protein NeuN (*RBFOX3*), by the $2^{-\Delta\Delta ct}$ method. In addition, *RBFOX3* levels were normalised to ubiquitous house-keeping gene *GAPDH* to assess any influence of PMD on gene expression.

Genotyping

Extracted gDNA was genotyped using qPCRBIO Genotyping Mix Hi-ROX (PCR Biosystems; PB20.42-05) and inventoried TaqMan® SNP Genotyping Assays (ThermoFisher Scientific; 4351379) for *rs8702 (C__11467163_10)*, *rs8007903 (C___2169408_10)*, *rs12368653* (C__26237749_10) and *rs4646536* (C__25623453_10) (proxy for *rs703842*), according to the manufacturer's instructions. Genotyping was performed on a StepOnePlus™ Real-Time PCR system with StepOne software v2.1 (ThermoFisher Scientific), on a FAST ramp speed (Holding at 60°C for 30 s, 95°C for 3 min, cycling (x40) at 95°C for 15 s, 60°C for 1 min, holding at 60°C for 30 s).

Western blot

Validation of primary antibody specificity for use in dot blot analysis was verified using western blot, as per previous studies[25]. Protein homogenates were diluted 1:1 with Laemmli 2x sample buffer (Sigma-Aldrich Ltd; S3401) and heated to 95°C to denature the protein. Samples and BLUeye Prestained Protein Ladder (Geneflow; S6-0024) were loaded onto mini-Protean TGX gels (4–20%) (Biorad; 456-1093) in a Mini-PROTEAN Tetra cell (Biorad; 1658004), filled with Tris/Glycine/SDS running buffer (Biorad; 161-0732). The gel was run at 150 V until the dye front reached the bottom of the gel, before protein transfer onto nitrocellulose membrane for 90 min at 350 mA. Membranes were blocked in 5% BSA/Tris-buffered saline-Tween 20 (TBS-T) or 5% milk/TBS-T, for 1 h at room temperature. Primary antibodies were reconstituted in membrane blocking solution (detailed in Table 2) and added overnight at 4°C. The primary antibodies used were: mouse anti-AβPP (ThermoFisher Scientific; 13-0200), mouse anti-GAPDH (Abcam; Ab9484); mouse anti-PHF-TAU (ThermoFisher Scientifc UK; MN1020), rabbit anti-KIF5A (Sigma-Aldrich Ltd; HPA004469), rabbit anti-KLC1 (Abcam; Ab174273) and rabbit anti-NeuN (Abcam; Ab177487). Bound primary antibody was detected by incubation with HRP-conjugated secondary antibodies for 1 h at room temperature (as detailed in Table 2). Protein expression was visualised using a chemiluminescence Clarity™ Western ECL Substrate (Biorad; 1705060). Antibodies displayed bands relative to their reported molecular weight, as described on manufacturer data sheets (Figure 1).

Table 2. Antibodies used for immunoblotting.

Antigen	Species	Blocking antibody	WB/DB concentration	Company	RRID	HRP-conjugated secondary	RRID
APP	Mouse monoclonal	5% milk/TBS-T	1:500	ThermoFisher Scientific; 13-0200	AB_2532993	Goat anti-mouse IgG 1:5000 Abcam; Ab6789	AB_955439
GAPDH	Mouse monoclonal	5% milk/TBS-T	1:5000	Merck; MAB374	AB_2107445		
PHF-Tau	Mouse monoclonal	5% BSA/TBS-T	1:500	ThermoFisher Scientific; MN1020	AB_223647	Goat anti-mouse IgG 1:10,000 Stratech; 115-035-146-JIR	AB_2307392
KIF5A	Rabbit polyclonal	5% milk/TBS-T	1:1000	Sigma-Aldrich; HPA004469;	AB_1079212	Goat anti-rabbit IgG 1:10,000 Abcam; ab7090	AB_955417
KLC1	Rabbit monoclonal	5% BSA/TBS-T	1:5000	Abcam; Ab174273	AB_2783556		
NeuN	Rabbit monoclonal	5% milk/TBS-T	1:5000	Abcam; Ab177487	AB_2532109		

Abbreviations: APP: amyloid precursor protein; BSA: bovine serum albumin; DB: dot blot; KIF: kinesin superfamily protein; KLC1: kinesin light chain-1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; NeuN: neuronal nuclear protein; PHF: paired-helical filament; RRID: Research Resource Identifiers; T: TBS-T: tris-buffered saline-tween; WB: western blot.

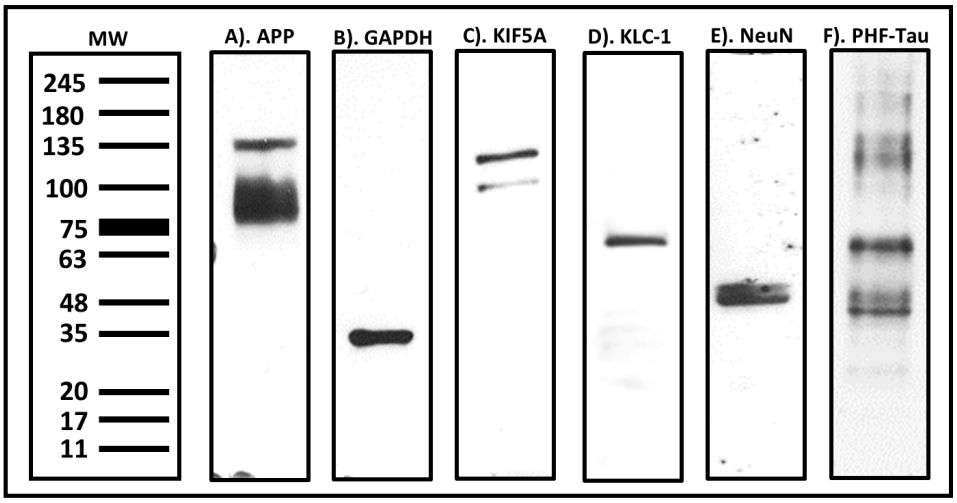

Figure 1. Antibody specificity. western blots performed using AD protein homogenates, derived from mid-frontal cortex. Predicted molecular weight of APP 110-135kDa. Antibody used for detection was mouse anti-APP (1:2000), ThermoFisher Scientific; 13-0200 (**A**). Predicted molecular weight of GAPDH 37kDa. Antibody used for detection was mouse anti-GAPDH (1:5000), Merck; MAB374 (**B**). Predicted molecular weight of KIF5A 107kDa and 117kDa. Antibody used for band detection was rabbit anti-KIF5A (1:1000), Sigma-Aldrich; HPA004469 (**C**). Predicted molecular weight of KLC1 60-70kDa. Antibody used for detection was rabbit anti-KLC1 (1:5000), Abcam; Ab174273 (**D**). Predicted molecular weight of NeuN 48kDa. Antibody used for detection was rabbit anti-NeuN (1:5000), Abcam; Ab177487 (**E**). Predicted molecular weight of paired-helical filament tau 45-65kDa. Antibody used for detection were mouse anti-PHF-Tau (1:500), ThermoFisher Scientific; MN1020 (**F**). APP: amyloid precursor protein; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; kDa: kilodaltons; KIF: kinesin superfamily motor protein; KLC1: kinesin light chain-1; MW: molecular weight; PHF: paired helical filament; NeuN: neuronal nuclear protein.

Dot blot

Dot blot was performed using a Bio-Dot Microfiltration manifold (Biorad), according to the manufacturer's instructions. Nitrocellulose membrane was pre-soaked in 1x TBS and placed in the manifold, before addition of 100 µL of protein homogenate/well (diluted 1:200 with 1x TBS). After microfiltration for 90 min, the membrane was removed and placed in blocking antibody, before antibody incubation (Table 2), as per the western blot protocol. Protein expression was visualised on a Biorad Universal III Bioplex imager, using a chemiluminescence Clarity™ Western ECL Substrate (Biorad). Image Lab™ 5.0 software (Biorad) was used for densitometric protein analysis. Integrated density values were expressed relative to the neuronal nuclear protein NeuN. In addition, NeuN protein levels were normalised to ubiquitous protein GAPDH to study any effect of PMD on protein expression.

Enzyme-linked immunosorbent assay (ELISA)

AD frontal tissue (200 mg) was homogenised in TBS extraction buffer to obtain the soluble protein fraction and the protein pellet was homogenised in guanidine to obtain the insoluble fraction, as previously described[25,30]. Sandwich ELISA was used to measure total Aβ in the fractions, with mouse monoclonal anti-Aβ (4G8 clone) (Biolegend; 800712; RRID: AB_2734548), for the capture step and biotinylated anti-human Aβ monoclonal antibody (10H3 clone) (ThermoFisher Scientific; MN1150B; RRID: AB_223641), for the detection step, as previously described[31].

Immunoperoxidase staining of paraffin sections

7 µm sections from frontal cortex were dewaxed, hydrated, and immersed in 3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. Sections were subsequently rinsed in running tap water and pre-treated with sodium citrate buffer in the microwave (0.01 M, pH 6.0, 5 min) to unmask antigenic sites, then rinsed in PBS. Immunostaining was performed using VECTASTAIN® Universal Elite® ABC Kit (Vector Laboratories; PK-6200). Non-specific binding was blocked using horse serum for 30 min at room temperature, before incubation overnight at 4°C with anti-mouse monoclonal PHF-tau (1:2000) (ThermoFisher Scientific; MN1020; RRID: AB_223647), diluted in PBS. Sections were rinsed in PBS and incubated for 20 min with VECTASTAIN® biotinylated universal secondary antibody, followed by 20 min with VECTASTAIN® Elite® ABC complex (both Vector Laboratories; PK-6200), before a final 7 min with 3,3'-diaminobenzidine (DAB) (Vector Laboratories; SK4100). Sections were washed in water, immersed in copper sulphate DAB enhancer for 4 min (0.16 M Copper (II) sulphate 5-hydrate/0.12 M sodium chloride) and counterstained with Gills haematoxylin II (Sigma-Aldrich Ltd; GHS216) for 15 s. Sections were subsequently dehydrated, cleared and mounted in Clearium® (Leica Biosystems; 3801100). Controls in each run included sections incubated overnight in PBS instead of the primary antibody.

Immunohistochemical analysis

The percentage field fraction immunopositive for PHF-tau (insoluble tau load) was assessed in the cortex of frontal sections from AD and controls by computer-assisted image analysis using Histometrix software (Kinetic Imaging, Nottingham, UK) driving a Leica DM microscope with a motorised stage, as previously described[32,33]. The area to be analysed was selected at low-magnification (x2.5) and the threshold for PHF-tau labelling density calibrated at high magnification (x20). The

software was programmed to measure tau load in 30 random regions (x20 objective fields) within the selected area and determine the cumulative area fraction with a density exceeding the threshold value.

Statistical analysis

Univariate mRNA and protein analysis were performed using GraphPad Prism 5™ (GraphPad Software Inc.; San Diego, USA). Data normality was tested using the Shapiro-Wilk test. One-way ANOVA with post-hoc Bonferroni, or Kruskal-Wallis with post-hoc Dunns test, as appropriate, was used to analyse differences in mRNA and protein expression according to Braak stages and SNP genotype. Parametric Pearson's or non-parametric Spearman's correlation was used to assess any relationship between proteins. A multiple regression model (STATA v12; StataCorp LLC; Texas, USA) was used to analyse mRNA expression in relation to Braak stage pathology, patient age of death and tissue post-mortem delay. Where necessary, data were transformed to normality before regression analysis. For all tests, values of $p < 0.05$ were considered statistically significant.

Results

Cohort variables

With exclusion criteria, the study cohort comprised 47 AD cases and 39 controls, as detailed in Table 1. The age of AD cases ranged from 54-98 y (mean 81, SD 9). Control cases ranged from 43-95 y (mean 80, SD 11). There were more females (n = 29) than males (n = 18) in the AD group, and fewer females (n = 15) than males (n = 24) in the controls. The PMD did not differ significantly between AD (37 h, SD 19) and controls (34 y, SD 20; two-tailed Mann-Whitney p = 0.34). There was no influence of PMD on *RBFOX3* (NeuN) mRNA (normalised to *GAPDH*; n =70, Spearman r = -0.09, p = 0.47) or protein level (normalised to GAPDH; n = 79, Spearman r = -0.02, p = 0.87).

Significant increase in kinesin gene expression linked to AD pathology

Expression of kinesin relative to *RBFOX3* mRNA was measured by qPCR and analysed in relation to Braak tangle stage. *KIF5A* mRNA was significantly elevated at Braak stages III-IV ($p < 0.01$) and V-VI ($p < 0.001$), compared to stages I-II, (Figure 2A). *KLC1* mRNA was significantly elevated in late Braak stage disease (V-VI, $p < 0.001$) compared to stages I-II (Figure 2B). In a previous study we found an association between PMD and the level of *KIF5A* relative to *RBFOX3* (NeuN) mRNA[25] but in the present cohort we found no effect of either patient age at death or PMD on expression of *KIF5A* or *KLC1* relative to *RBFOX3* (Table 3).

Single nucleotide polymorphisms within the KIF5A gene locus influence KIF5A mRNA expression

We previously showed that KIF5A protein level was reduced in post-mortem brain tissue from MS patients homozygous for SNPs within the *KIF5A* gene locus which have been linked to MS susceptibility[24]. We explored this in AD brain tissue using pre-designed TaqMan® SNP assays for *rs12368653* and *rs4646536*, the latter in linkage disequilibrium ($r^2 = 1$) with *rs703842,* making it suitable as a proxy[34,35]. The accuracy of the SNP was verified using 18 MS positive control genotypes from our previous studies[24].

KIF5A mRNA expression was significantly lower in AD patients homozygous for the *rs12368653* SNP (AA) than in AD patients with no copies (GG) ($p<0.05$; Figure 3A). Similarly, AD patients homozygous for the *rs4646536* SNP (AA) (proxy for *rs703842*), had significantly lower *KIF5A* mRNA than did heterozygous patients (AG) ($p<0.05$; Figure 3B). There was no significant difference in *KLC1* mRNA expression in AD patients stratified according to KIF5A SNPs (*rs12368653* and *rs4646536*; Figure 3C and 3D). We subsequently investigated whether SNPs in the *KLC1* gene that were previously linked to AD

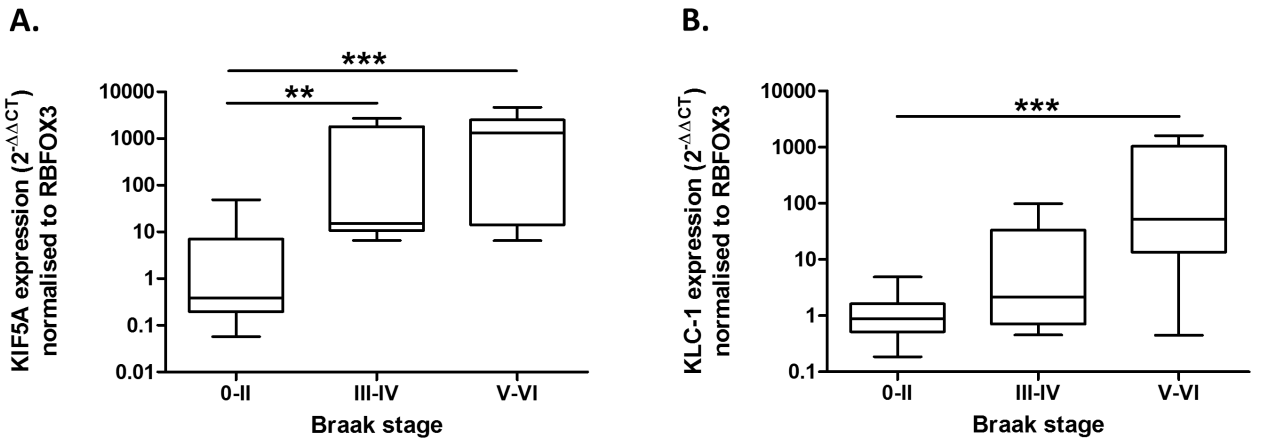

Figure 2. Upregulation of kinesin mRNA in Alzheimer's disease. Quantitative real-time PCR on cDNA from mid-frontal cortex of control and AD cases showed a significant increase in *KIF5A* mRNA in Braak stages III-IV (n=11) and V-VI (n=35) compared with Braak stages 0-II (n=27) (**A**). *KLC1* mRNA was significantly increased in Braak stages V-VI (n=28) but not stages III-IV (n=12), compared with Braak stages 0-II (n=31) (**B**). Results expressed as median, IQR and min/max quartile. Statistical test used: Kruskal-Wallis with post-hoc Dunns (**A**–**B**), **p<0.01, ***p<0.001. AD: Alzheimer's disease, IQR: inter-quartile range, KIF: kinesin superfamily protein, *KLC1*: kinesin light chain-1, *RBFOX3*: RNA Binding Fox-1 Homolog 3.

Table 3. Multiple regression analysis of cohort variables on kinesin mRNA expression.

Sample type	n	Variables	Coefficient	Standard error	t	p value	95% Confidence intervals	
KIF5A to *RBFOX3* mRNA	72	*Braak III-IV*	16.14	6.08	2.66	0.01	4.01	28.27
		Braak V-VI	31.12	3.90	7.98	0.00	23.33	38.91
		Age at death	0.08	0.20	0.40	0.69	-0.33	0.49
		Post-mortem delay	0.02	0.10	0.15	0.88	-0.19	0.22
KLC1 to *RBFOX3* mRNA	70	*Braak III-IV*	-0.35	0.17	-2.04	0.05	-0.69	-0.01
		Braak V-VI	-0.77	0.12	-6.43	0.00	-1.01	-0.53
		Age at death	-0.00	0.01	-0.69	0.50	-0.02	0.01
		Post-mortem delay	-0.00	0.00	-0.09	0.93	-0.01	0.00

Significant correlations highlighted in red. Abbreviations: KIF: kinesin superfamily protein; *KLC1*: kinesin light chain-1; *RBFOX3*; RNA Binding Fox-1 Homolog 3.

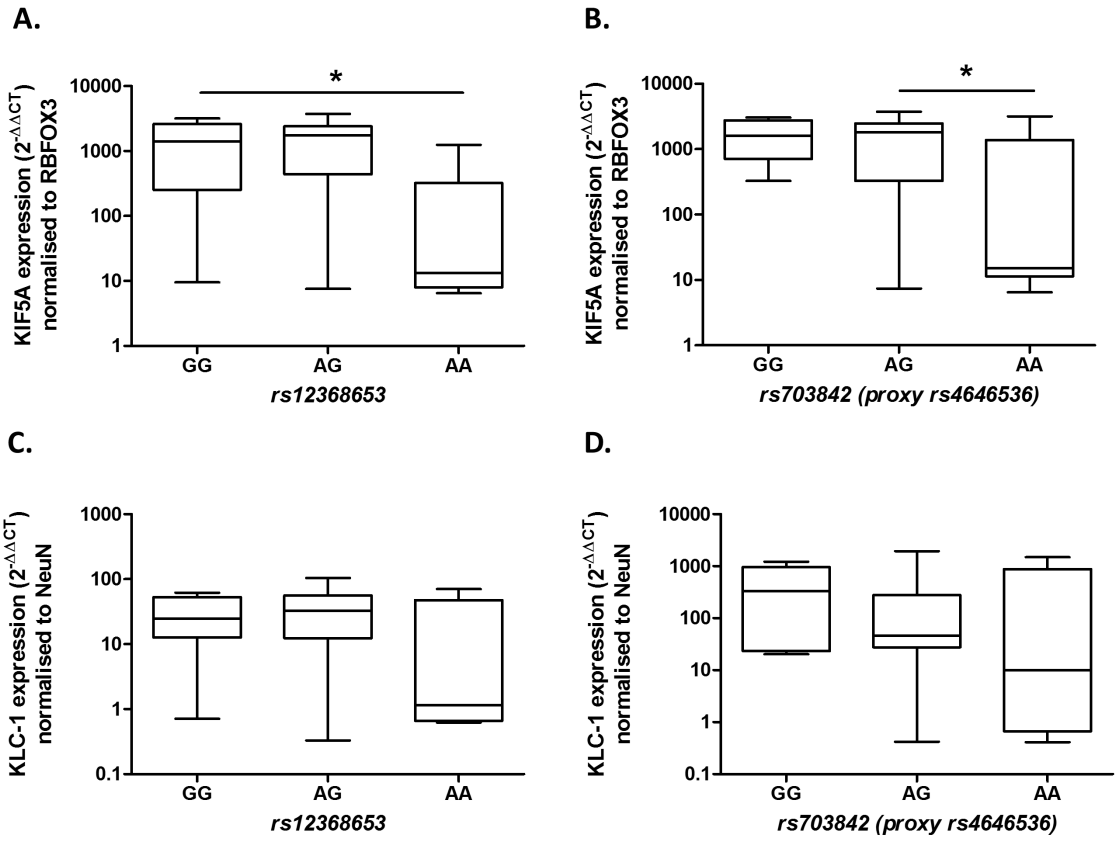

Figure 3. Significant reduction in *KIF5A* mRNA expression in patients homozygous for SNPs within *KIF5A* gene locus. Homozygous AD carriers of *rs12368653* 'A' risk allele, (linked to MS susceptibility), had significantly lower levels of *KIF5A* mRNA (AA; n=6) than did non-carriers (GG; n=10) (**A**). Homozygous AD carriers of *rs703842* 'A' risk allele, (identified by proxy SNP *rs4646536*), also linked to MS susceptibility showed significant reduction in *KIF5A* mRNA (AA; 18) compared to expression in heterozygous carriers (AG; n=17) (**B**). No significant difference in *KLC1* mRNA between homozygous (AA; n=6) or heterozygous (AG; n=22) AD carriers of *rs12368653* 'A' risk allele, (linked to MS susceptibility), compared to non-carriers (GG; n=8) (**C**). Homozygous (AA; n=17) or heterozygous (AG; n=14) AD carriers of *rs703842* 'A' risk allele, (identified by proxy SNP *rs4646536*), also linked to MS susceptibility, showed no significant difference in *KLC1* mRNA compared to non-carriers (GG; n=5) (**D**). Results expressed as median, IQR and min/max quartile. Statistical test used for **A** and **C**: one-way ANOVA, post-hoc Bonferroni Multiple Comparison. Statistical test used for **B** and **D**: Kruskal-Wallis test, post-hoc Dunn's Multiple Comparison; * p<0.05. AA: homozygous adenine, AD: Alzheimer's disease, AG: adenine/guanine, GG: homozygous guanine, IQR: inter-quartile range, KIF: kinesin superfamily protein, *KLC1*: kinesin light chain-1, MS: multiple sclerosis, *RBFOX3*: RNA Binding Fox-1 Homolog 3, SNP: single nucleotide polymorphism.

susceptibility[21,22] influence kinesin mRNA expression. There was no significant difference in *KLC1* mRNA between AD patients heterozygous for the *rs8702* SNP (GC) and those with no copies (GG; Figure 4A). Similarly, there was no significant difference in *KLC1* mRNA between AD patients heterozygous for the *rs8007903* SNP (AG) and those with no copies (AA; Figure 4B). Due to low cases numbers (n=2), homozygous expression could not be analysed. There was no significant effect of *KLC1* SNPs on *KIF5A* mRNA expression (Figure 4C and 4D).

Kinesin protein level does not vary significantly with AD pathology

Kinesin protein expression was measured by dot blot. In keeping with previous studies[25], KIF5A expression did not differ significantly between Braak stages 0-II, III-IV and V-VI (Figure 5A). In addition, there was no significant variation in KLC1 protein level with Braak stage (Figure 5B). As expected, the levels of KIF5A and KLC1 protein were positively correlated ($p < 0.001$, Figure 5C). There was no influence of SNPs within the KIF5A gene locus (*rs12368653* and *rs4646536*) on KIF5A (Figure 6A and 6B) or KLC1 protein level (Figure 6C and 6D).

Kinesin levels inversely correlate with amyloid precursor protein in AD

As in previous studies[25,36], APP level did not differ significantly between AD and control cases (Figure 7A). APP levels correlated inversely with both KIF5A and KLC1 protein in AD ($p < 0.05$; Figure 7B and 7C). Neither KIF5A or KLC1 correlated with the level of soluble or insoluble Aβ or with the ratio of soluble: insoluble Aβ (Table 3), although a trend was seen for lower levels of soluble Aβ to be associated with higher levels of KIF5A (p=0.10; Table 3), as in our previous study[25]. As expected, soluble and insoluble Aβ levels were significantly higher in AD than controls (Figure 8A and 8B).

KIF5A protein level correlates inversely with soluble paired helical filament tau

The level of hyperphosphorylated tau in the soluble protein fraction was significantly increased in AD compared with control cases (Figure 9A). The level correlated inversely with KIF5A protein level ($p<0.05$) but not with KLC1 protein in AD (Figure 9B and 9C). Western blot analysis showed additional bands above the predicted molecular weight of tau (45-65kDa),

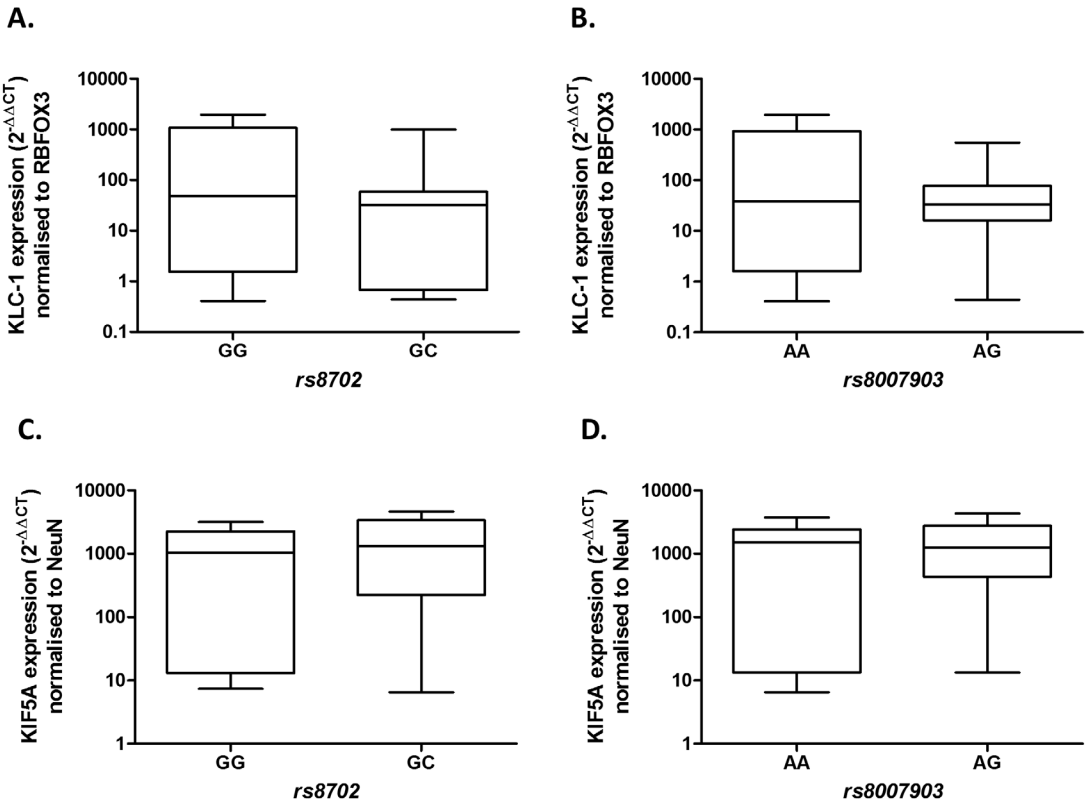

Figure 4. No effect of SNPs within the *KLC1* gene on kinesin mRNA expression. No difference in *KLC1* mRNA between heterozygous carriers of *rs8702* 'C' risk allele linked to AD susceptibility (GC; n=14) and non-carriers (GG; n=21) (**A**). No difference in *KLC1* mRNA between heterozygous carriers of *rs8007903* 'G' risk allele linked to AD susceptibility (AG; n=7) and non-carriers (AA; n=26) (**B**). No difference in *KIF5A* mRNA between heterozygous carriers of *rs8702* 'C' risk allele linked to AD susceptibility (GC; n=17) and non-carriers (GG; n=24) (**C**). No difference in *KIF5A* mRNA between heterozygous carriers of *rs8007903* 'G' risk allele linked to AD susceptibility (AG; n=9) and non-carriers (AA; n=29) (**D**). Results expressed as median, IQR and min/max quartile. Statistical test used: two-tailed Mann-Whitney. AA: homozygous adenine, AD: Alzheimer's disease, AG: adenine/guanine, GC: guanine/cytosine, GG: homozygous guanine, IQR: inter-quartile range, KIF: kinesin superfamily protein, *KLC1*: kinesin light chain-1, MS: multiple sclerosis, *RBFOX3*: RNA Binding Fox-1 Homolog 3, SNP: single nucleotide polymorphism.

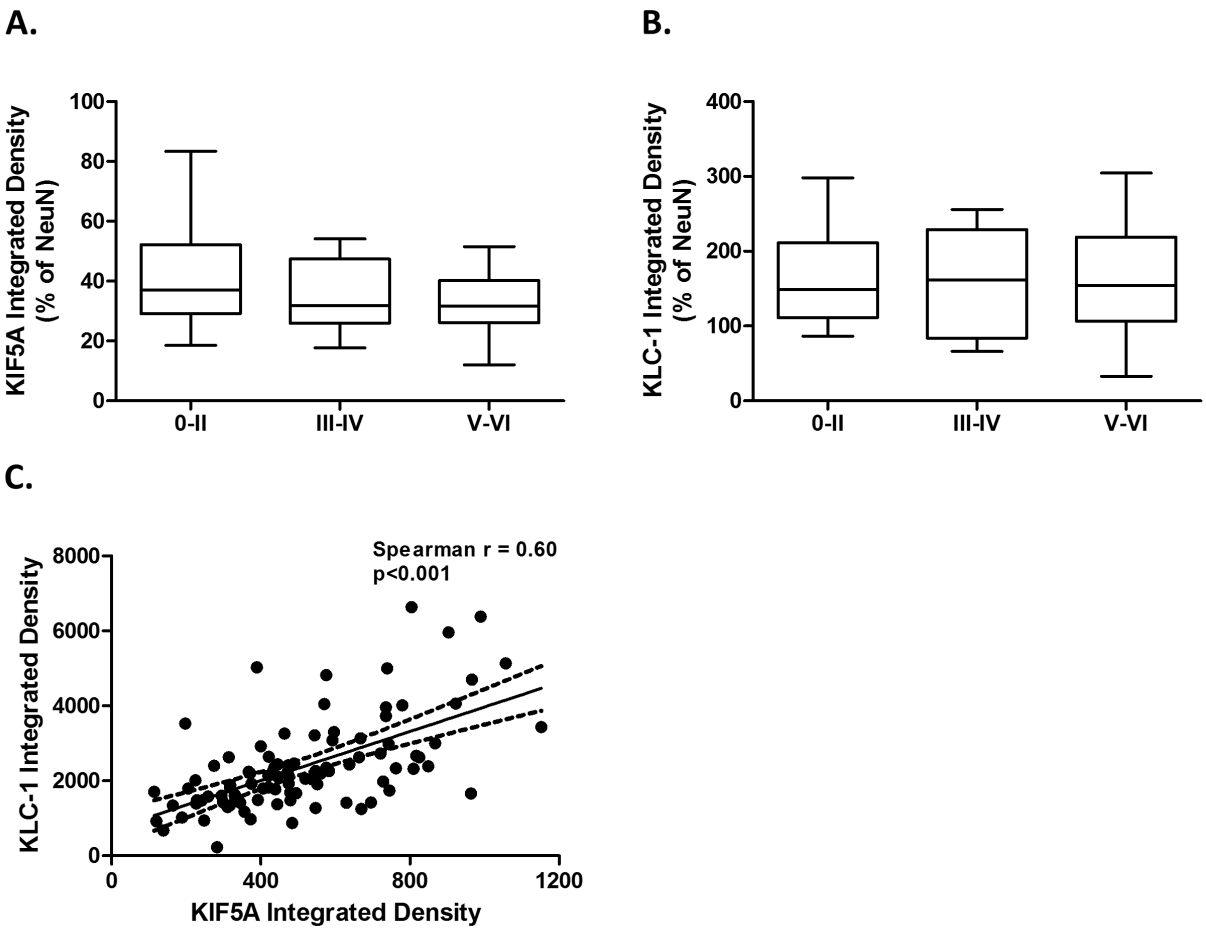

Figure 5. No change in conventional kinesin expression in Alzheimer's disease. No significant difference in KIF5A between Braak stages 0-II (n=29), III-IV (n=12) and V-VI (n=34), on dot blot analysis of protein extracted from homogenised control and AD mid-frontal cortex (**A**). No difference in KLC1 protein between Braak stages 0-II (n=28), III-IV (n=11) and V-VI (n=35) (**B**). Protein levels of KIF5A correlated positively with that of KLC1 (n=86; **C**). Results expressed as median, IQR and min/max quartile. Statistical test used: Kruskal-Wallis test, post-hoc Dunn's Multiple Comparison (**A**–**B**). Spearman correlation represented as line of best fit +/- 95% CI (**C**); ***p<0.001. AD: Alzheimer's disease, CI: confidence intervals, IQR: inter-quartile range, KIF: kinesin superfamily protein, KLC1: kinesin light chain-1, NeuN: neuronal nuclear protein.

most likely attributed to phosphorylated epitopes[37]. In addition to immunoblotting, hyperphosphorylated tau levels were measured by quantitative immunohistochemistry and showed a significant positive correlation with hyperphosphorylated tau protein levels measured by dot blot (p<0.001; Figure 9D). There was no correlation between KIF5A or KLC1 protein level and insoluble tau load (Table 4).

Discussion

Previous studies in mouse models implicated defective functioning of KLC1 in disruption of axonal transport in AD[19,38]. In the current study of human tissue from AD and control cases, we have found *KLC1* and *KIF5A* gene expression to be elevated in AD and associated with Braak tangle stage. We have also demonstrated significant inverse correlations between kinesin levels and AD-associated proteins, emphasising the importance of kinesins in AD pathology. We did not find any association between *KLC1* gene expression and *KLC1* SNPs reportedly linked to AD.

Four KLC genes are expressed in mammals but those involved in axonal transport are *KLC1* and *KLC2*. KLC1 is neuronally enriched whereas KLC2 is ubiquitously expressed[39]. A coiled-coil region at the amino terminus of the KLCs binds to the stalk domain (close to the carboxyl terminus) of conventional kinesins (KIF5A, KIF5B and KIF5C) and acts as an adaptor complex for indirect binding and transport of cellular cargoes, such as APP[27].

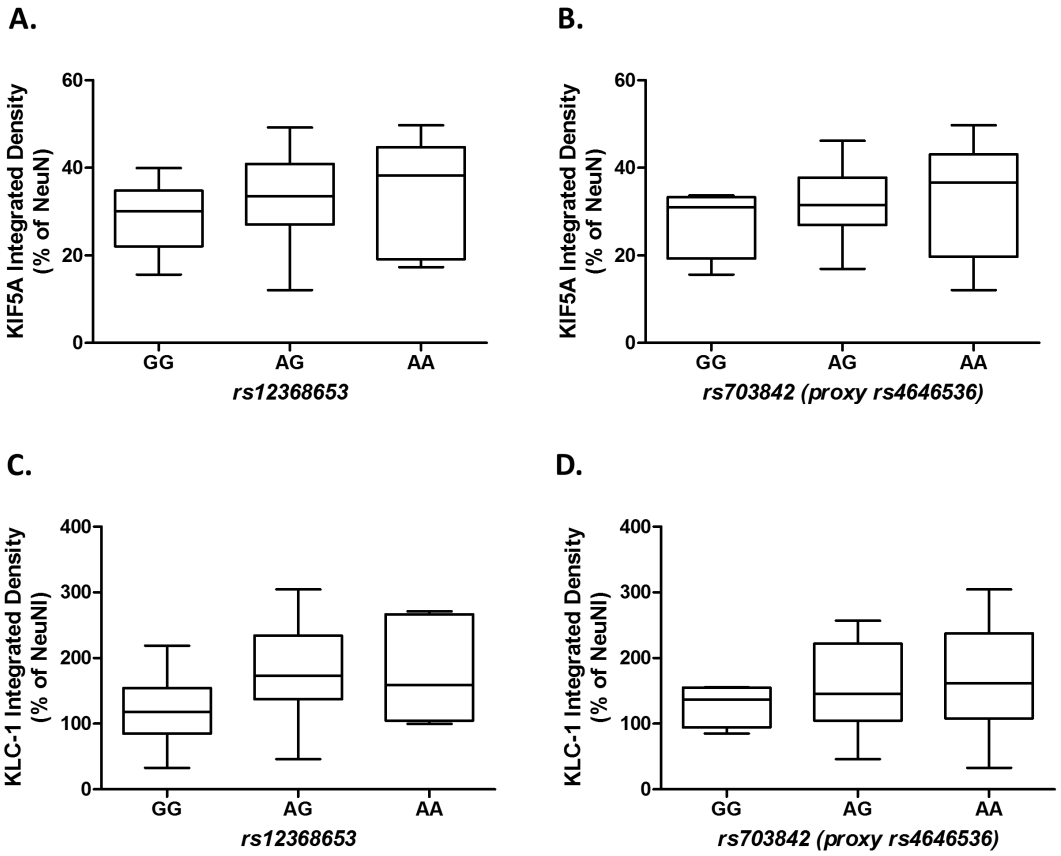

Figure 6. Kinesin protein expression in AD patients homozygous for SNPs within the *KIF5A* gene locus. No difference in KIF5A protein between homozygous AD carriers of *rs12368653* 'A' risk allele, linked to MS susceptibility, (AA; n=9) and heterozygotes (AG; n=21) or non-carriers (GG; n=6) (**A**). No difference in KIF5A protein between homozygous AD carriers of *rs703842* 'A' risk allele, (identified by proxy SNP *rs4646536*) linked to MS susceptibility, (AA; 15) and heterozygotes (AG; n=17) or non-carriers (GG; n=4) (**B**). No difference in KLC1 protein between homozygous AD carriers of *rs12368653* 'A' risk allele, linked to MS susceptibility, (AA; n=6) and heterozygotes (AG; n=21) or non-carriers (GG; n=10) (**C**). No difference in KLC1 protein between homozygous AD carriers of *rs703842* 'A' risk allele, (identified by proxy SNP *rs4646536*) linked to MS susceptibility, (AA; 17) and heterozygotes (AG; n=17) or non-carriers (GG; n=5) (**D**). Results expressed as median, IQR and min/max quartile. Statistical test used: one-way ANOVA, post-hoc Bonferroni Multiple Comparison. AA: homozygous adenine, AD: Alzheimer's disease, AG: adenine/guanine, GG: homozygous guanine, IQR: inter-quartile range, KIF: kinesin superfamily protein, KLC1: kinesin light chain-1, MS: multiple sclerosis, NeuN: neuronal nuclear protein.

Expression of both *KLC1* and *KIF5A* mRNA was positively associated with Braak tangle stage, a widely used marker of AD progression, defined by the distribution of hyperphosphorylated tau pathology distribution within the brain[40]. *KIF5A* mRNA was upregulated at relatively early stages of disease (Braak stages III-IV), in keeping with previously reported evidence of early-stage axonal abnormalities in AD and other neurodegenerative diseases[13,19,38]. Upregulation of both *KIF5A* and *KLC1* mRNA may be a compensatory response to other pathological processes that interfere with axonal transport of protein cargoes to the synapse. Elevated kinesin gene expression in human tissue has been demonstrated in several neurodegenerative diseases[15,26,41].

Genome-wide association studies (GWAS) have shown linkage of multiple genes to AD susceptibility, including some genes encoding proteins with roles in axonal transport and cytoskeletal function (*NME8*, *CELF1*, and *CASS4*)[3]. However, GWAS are unable to detect rare variants linked to susceptibility. Such variants include SNPs within the *KLC1* gene, which have been linked to early AD pathogenesis. In particular, *rs8702* was reported to predict conversion from mild cognitive impairment to AD and was associated with levels of hyperphosphorylated tau in patient cerebrospinal fluid[16]. It is hypothesised that this SNP could influence the splicing of c-terminal epitopes in *KLC1* gene transcripts[16], which could affect cargo binding affinity, axonal transport and synaptic function, causing structural instability and eventual neuronal degeneration. The effective 'strain' on the neuron caused by transport defects is likely to be exacerbated by a reduction in mitochondrial ATP supply associated with aging[42]. We found no effect on *KLC1* expression of heterozygosity of the *rs8702* 'C' risk allele or *rs8007903* 'G' allele but did not have sufficient cases to analyse the influence of homozygosity of the SNPs. Larger genetic studies found no effect of *rs8702* on AD

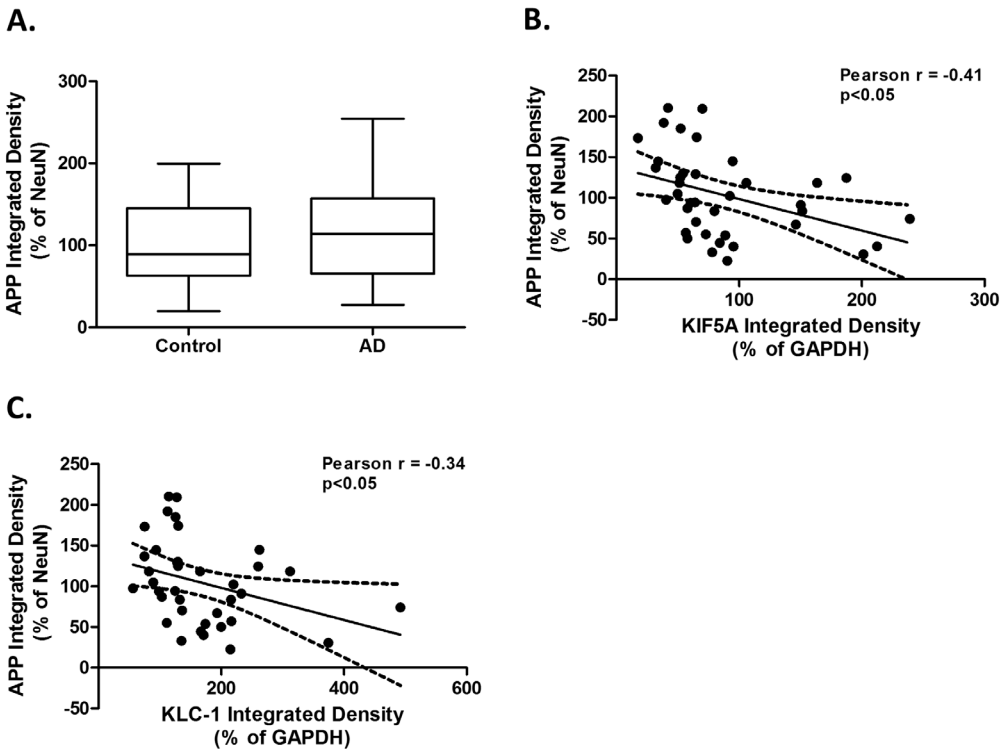

Figure 7. Kinesin levels correlate inversely with APP expression in AD. No significant difference in APP between control (n=36) and AD cases (n=40) on dot blot analysis of protein extracted from homogenised control and AD mid-frontal cortex (**A**). Significant inverse correlation between KIF5A (normalised to GAPDH) and APP (normalised to NeuN) protein levels in AD (n=38; **B**). KLC1 level (normalised to GAPDH) correlated inversely with APP level (normalised to NeuN) in AD cases (n=37; **C**). Results expressed as median, IQR and min/max quartile. Statistical test used two-tailed t-test (**A**). Pearson correlations represented as line of best fit +/- 95% CI (**B**–**C**); *p<0.05. AD: Alzheimer's disease, APP: amyloid precursor protein, CI: confidence intervals, IQR: inter-quartile range, KIF: kinesin superfamily protein, KLC1: kinesin light chain-1, NeuN: neuronal nuclear protein.

susceptibility but a significant interaction with *APOE* ε4 carrier status in patients[16]. The continuing uncertainty as to the influence of *KLC1* polymorphisms on the risk of AD highlights the difficulties of linkage studies for rare variants, which are often hampered by small sample size, locus heterogeneity and false-positive results[3].

We previously found a significant reduction in KIF5A expression in MS tissue in homozygous carriers of the *rs703842* 'A' allele and *rs12368653* 'A' allele[23,24], which are linked to MS susceptibility[34,43]. GWAS have identified susceptibility variants common to MS and sporadic AD, including major histocompatibility complex gene *HLA-DRB1*[3]. In the present study, we found a significant reduction in *KIF5A* mRNA expression in AD patients homozygous for the *rs4646536* 'A' allele (proxy for *rs703842*[35]) and *rs12368653* 'A' allele, compared to heterozygotes and non-carriers. Variability within the KIF5A gene locus may be a common denominator across a spectrum of neurodegenerative conditions. KIF5A mutations are linked to several axonopathies, including hereditary spastic paraplegia and Charcot-Marie Tooth Disease[44,45]. More recently, a mutation in the cargo binding domain of *KIF5A* was shown to be associated with amyotrophic lateral sclerosis[46]. These findings highlight the importance of KIF5A for axonal transport and maintenance of neuronal function and integrity.

Unlike our findings in MS, although the *KIF5A* genotype influenced mRNA expression in AD it did not have a detectable effect on expression of the protein. We previously found increased KIF5A protein in AD, although no significant association with Braak stage[25]. Similarly, in the present study we found no significant association between KIF5A or KLC1 and Braak stage. It is possible that reduced protein clearance, a common finding in age-related neurodegenerative diseases[47], could partly compensate for reduced KIF5A synthesis in the later stages of AD. Aβ itself may influence kinesin metabolism. Previous studies found reduced KLC1 protein in AD frontal cortex[48] and reduced KLC1 expression in the presence of soluble Aβ trimers[49]. Assay sensitivity may also be relevant and some changes in KLC1 metabolism missed due to the complex carboxyterminal splicing, which results in multiple variants[50] with varied affinities for the antibody used in the present study. Co-immunoprecipitation studies have demonstrated at least 6 variants of conventional kinesin (KIF- 5A/5B/5C with KLC1 or KLC2); however, with alternative splicing of KLC1 the number is likely to be much higher[28]. The multiplicity of splice variants is attributed to the large variety of membrane cargoes that require transportation to distinct neuronal compartments[1].

Irrespective of overall kinesin protein levels, evidence points to disruption of convention kinesin transport in AD, through

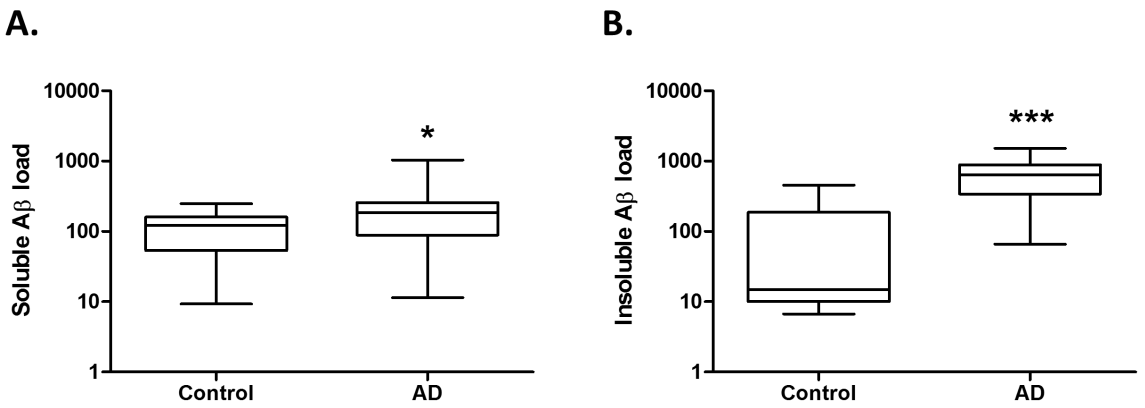

Figure 8. Expression of Aβ in mid-frontal cortex. Soluble Aβ significantly increased in AD cases (n=40) compared with control (n=18) from ELISA analysis (**A**). Insoluble Aβ significantly higher in AD cases (n=41) compared to control (n=23; **B**). Results expressed as median, IQR and min/max quartile. Statistical test used: two-tailed Mann-Whitney; **p<0.01. Aβ: amyloid beta, AD: Alzheimer's disease, IQR: inter-quartile range.

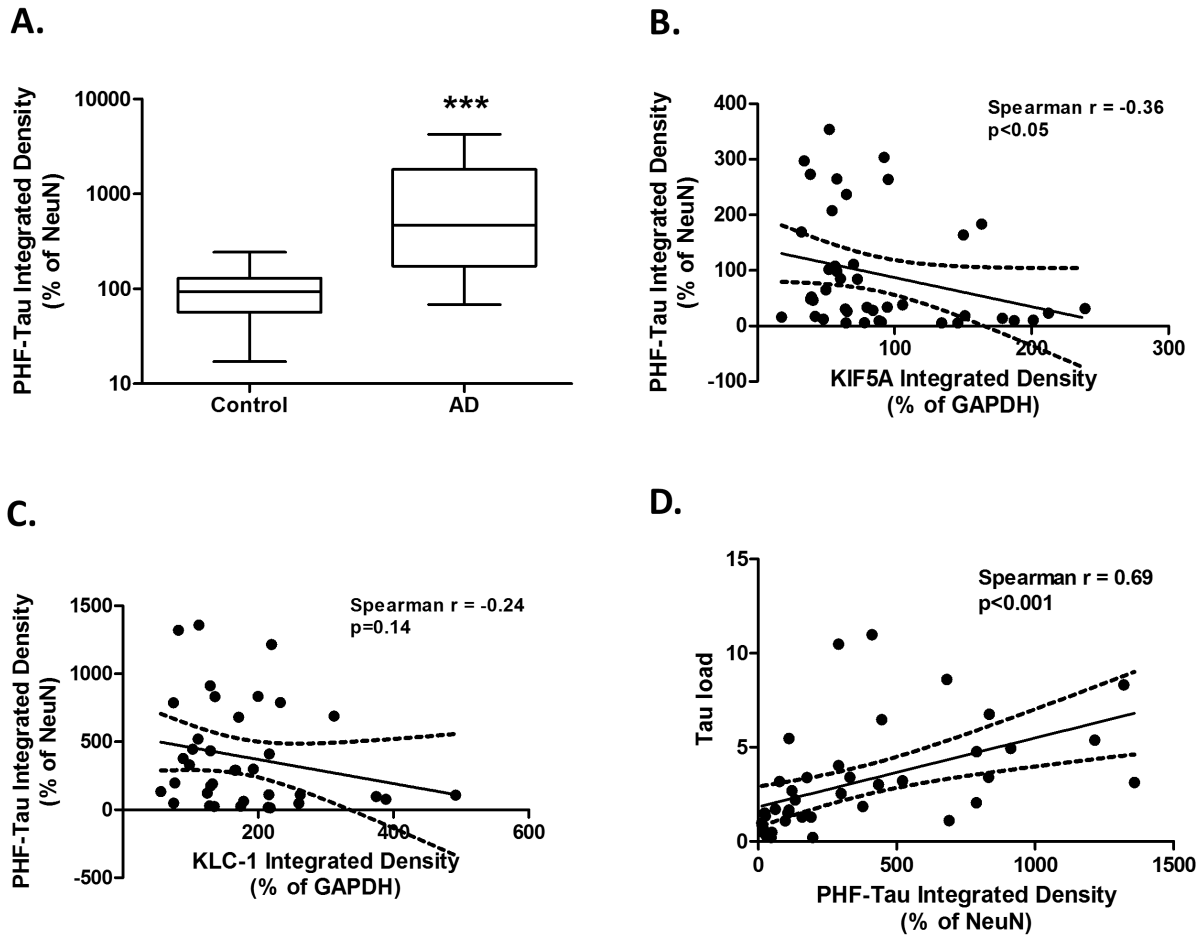

Figure 9. KIF5A levels correlate inversely with hyperphosphorylated tau expression in Alzheimer's disease. Significant increase in PHF-tau in AD cases (n=44) compared with control (n=35; **A**). Significant inverse correlation between KIF5A (normalised to GAPDH) and PHF-Tau (normalised to NeuN) in AD (n=44; **B**). No significant correlation between KLC1 levels (normalised to GAPDH) and PHF-Tau protein (normalised to NeuN) in AD (n=44; **C**). The level of soluble PHF-Tau (as measured by dot blot) showed a significant positive correlation with insoluble hyperphosphorylated tau load (as determined by IHC) in AD cases (n=44; **D**). Results expressed as median, IQR and min/max quartile. Statistical test used two-tailed Mann-Whitney (**A**). Spearman correlations represented as line of best fit +/- 95% CI (**B**–**D**); *p<0.05, ***p<0.001. AD: Alzheimer's disease, CI: confidence intervals, GAPDH: glyceraldehyde 3-phosphate dehydrogenase, IHC: immunohistochemistry, IQR: inter-quartile range, KIF: kinesin superfamily protein, KLC1: kinesin light chain-1, NeuN: neuronal nuclear protein, PHF: paired helical filament.

Table 4. Univariate correlation of kinesin and proteins associated with neuronal pathology.

Motor protein	Cargo protein	Sample	n	Correlation coefficient (r)	Significance (p)
KIF5A	*APP*	Control	33	-0.27	0.13
		AD	38	-0.41	0.01
	PHF-tau	Control	35	-0.17	0.35
		AD	44	-0.36	0.02
	Tau load	AD	42	0.10	0.52
	Insoluble Aβ	Control	22	0.25	0.25
		AD	37	-0.13	0.46
	Soluble Aβ	Control	19	0.14	0.58
		AD	37	-0.28	0.10
	Soluble: Insoluble Aβ	Control	14	-0.06	0.84
		AD	32	0.02	0.90
KLC1	*APP*	Control	34	-0.28	0.11
		AD	37	-0.34	0.04
	PHF-Tau	Control	33	-0.28	0.11
		AD	37	-0.24	0.14
	Tau load	AD	43	-0.05	0.73
	Insoluble Aβ	Control	22	0.29	0.20
		AD	39	0.03	0.88
	Soluble Aβ	Control	19	0.15	0.35
		AD	33	0.02	0.93
	Soluble: Insoluble Aβ	Control	14	-0.15	0.62
		AD	33	0.12	0.52

Significant correlations highlighted in red. Abbreviations: Aβ: amyloid beta; APP: amyloid precursor protein; KIF: kinesin superfamily protein; KLC1: kinesin light chain-1; PHF: paired helical filament.

phosphorylation-mediated dissociation of KIF from both its cargo and from the microtubule[51]. Studies of transgenic mouse models have suggested that Aβ can be transported within neurites and have shown a correlation between axonal swellings positive for intracellular Aβ and the location of extracellular insoluble Aβ deposits[20]. In addition, studies in the squid giant axon have shown that Aβ oligomers can inhibit axonal transport through activation of casein kinase 2, which phosphorylates KLC1, reducing its cargo binding affinity[52,53]. We found no significant correlation between KIF5A or KLC1 and insoluble Aβ levels, despite a trend towards lower soluble Aβ with higher KIF5A, as in our previous study[25]. There was a significant negative correlation between KIF5A and soluble hyperphosphorylated tau levels, but not with the insoluble tau load as determined immunohistochemically. Pathogenic forms of tau enhance glycogen synthase kinase 3β (GSK3β) activity by increasing exposure of the phosphatase-activating domain in the amino terminus of tau, which increases protein-phosphatase 1-dependent activation of GSK3β[54]. Elevated GSK3β causes KLC1 phosphorylation and results in protein cargo dissociation[38].

As kinesin phosphorylation is a potent modulator of axonal transport in neurodegenerative diseases, this pathway presents a potential modifiable target for treatment[1,55]. However, evidence suggests cargo transport is bi-directional in axons with both anterograde motor kinesin and retrograde motor dynein bound simultaneously in a 'tug-of-war' model, such that the loss or post-translation modification of one motor protein will affect motility in both directions[56,57]. Indeed, studies have reported reduced dynein intermediate chain in AD cortex[48]. In addition, protein kinases regulate a wide range of cellular activities, and for any attempted therapeutic interventions it will be important to establish the specificity of kinase inhibitors and phosphatases on kinesin expression.

Overall, the findings from this study do not indicate that upregulation of KIF5A is a result of functional redundancy between KIF5A and KLC1 in protein transport, or that the expression of KLC1 in cerebral cortex is influenced by *KLC1* polymorphisms, which have been linked to AD susceptibility. However, the findings highlight the importance of KIF5A in

maintaining axonal transport and raise the possibility that patients with higher 'reserve' levels of KIF5A are less susceptible to axonal transport disruption and pathology. The findings also suggest genetic polymorphisms within the *KIF5A* gene locus could represent a common neurodegenerative pathway across a spectrum of neurological conditions.

Data availability

The raw data files used for figure generation are available at the University of Bristol data repository, data.bris

Data.bris: Dataset 1. Data from BRACE pilot: axonal proteins (01-2019), https://doi.org/10.5523/bris.23clppyxx8fj92uf97zr2u41vc[58]

Immunohistochemical counts of insoluble tau load on AD cases were provided by the South West Dementia Brain Bank (SWDBB), as part of their archived data on Brain Bank cases. Due to the historic nature of the data set, the original IHC images are no longer available.

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Open Peer Review

Current Referee Status: ✗ ? ✓

Version 1

Referee Report 11 April 2019

https://doi.org/10.21956/amrcopenres.13927.r26330

✓ **Elizabeth Gray**
Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, University of Oxford, Oxford, UK

Hares et al. describe the expression of KLC1 and KIF5A gene and protein expression in the mid frontal cortex of Alzheimers Disease compared to healthy control patients. This study builds upon their body of work and observations of the kinesin expression in Multiple Sclerosis. The methodological approaches used are appropriate to address the questions being asked in the study and I would recommend this manuscript for indexing with the following addition to the discussion of the manuscript. Could the authors comment and perhaps discuss the relevance and potential merits of the use of live imaging of axonal transport in animal models of AD and how they may attempt to relate this to KIF5A and KLC protein expression in these models. Please see relevant references below.

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neurodegeneration, Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Neuropathology, Neurofilaments, Biomarkers.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 02 April 2019

https://doi.org/10.21956/amrcopenres.13927.r26328

? **Scott T. Brady**
Department of Anatomy and Cell Biology, University of Illinois at Chicago (UIC), Chicago, IL, USA

A substantial literature has implicated axonal transport deficits as a component of pathogenesis in AD and other adult-onset neurodegenerative diseases. Some evidence has been published from GWAS studies suggesting that altered expression of molecular motor proteins or genetic polymorphisms in KLC1 may be risk factors for AD, but relatively little has been done to document specific changes in patient materials. In this study, the investigators have evaluated expression of two genes that are candidates for a role in AD, taking advantage of access to midfrontal cortex tissue from 47 AD and 39 control human brains. The set of brains are relatively well matched in terms of mean age and postmortem delay for control and AD. Based on age at the time of death, the AD patients are likely to be mostly sporadic cases with 5-10% familial cases, which is consistent with the population levels. Both males and females are considered together. The authors do not stratify by familial vs sporadic or male vs female.

The focus here is on expression of KIF5A and KLC1, both of which have been reported to have polymorphisms associated with neurological diseases. In addition, the authors cite literature claiming that KIF5A complexed to KLC1 is responsible for transport of vesicles containing APP as well as b- and g-secretases. Unfortunately, those studies have never been replicated despite concerted effort by multiple groups (see for example *O. Lazarov, et al. (2007). Impairments in fast axonal transport and motor neuron deficits in transgenic mice expressing familial Alzheimer's disease-linked mutant presenilin 1*[1].). A more rigorous study of APP transport showed that the primary motor was KIF5C and that neither b- or g-secretases were co-transported (*A. Szodorai, et al. (2009). APP anterograde transport requires Rab3A GTPase activity for assembly of the transport vesicle*[2]). Nevertheless, evidence from this group and others that show increased expression of KIF5A in AD and data from GWAS and transcriptomic studies showing association of polymorphisms and splice variants of KLC1 with AD justify analysis of expression for these two genes.

The data showed an increase in mRNA for KIF5A confirming previous studies and indicate that KLC1 mRNA levels also increase. Data is also presented on changes in expression related to a polymorphism in KIF5A previously associated with reduced KIF5A and susceptibility to multiple sclerosis. While a small difference was seen with the AA genotype that was significant when analyzed by ANOVA, the effects were modest at best and were not compelling. No differences were detected with polymorphisms in KLC1. Similarly, protein levels of KIF5A and KLC1 were not different with either polymorphisms or Braak stage. The authors then looked at levels of Aband PHF-tau, finding that, of course, they both increase in the AD patients. Not surprisingly, the KIF5A levels correlated inversely with increased which is to be expected since KIF5A levels don't change while the Aband PHF-tau increase. Given the scatter of the

data, it is not clear that these are meaningful correlations.

In sum, this is a modest advance on previously published observations that largely yielded negative results. This may in part be due to the limited number of targets being investigated and questions as to whether they represent the right targets to evaluate. There are challenges associated with analyzing post-mortem human brains that inevitably exhibit substantial variants even in the most carefully executed studies. Unfortunately, even when sophisticated statistical tests are employed, the physiological relevance can be difficult to determine. This is particularly true when the absolute differences are small. This can be mitigated in part by using plots that show the actual data points (as in figures 5, 7 and 8), but the conclusions that can be drawn from this dataset are limited. Certainly, they do not provide much support for the overall conclusions. A more comprehensive study looking at changes at the full range of KIF5 and KLC forms would help as would comparisons to brain regions that are not associated with AD pathology (i.e. cerebellum and sensory cortex for example). However, in its present form, the impact of this study is limited.

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: cell and molecular neuroscience, axonal transport, and adult onset neurodegeneration.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 15 March 2019

https://doi.org/10.21956/amrcopenres.13927.r26309

Gustavo F. Gustavo
Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMECCONICETUniversidad Nacional de Córdoba, Córdoba, Argentina

In the present manuscript Kelly Hares and co-workers aimed (as stated by the authors) “to determine whether KLC1 levels are altered in AD, the relationship between the levels of KLC1 and KIF5A, and whether expression of KLC1 in the cerebral cortex is influenced by KLC1 polymorphisms, which have been linked to AD susceptibility.”

The authors determined the kinesin mRNA levels relative to RBFOX3 by qPCR, in relation to Braak tangle stage, in a cohort composed of 47 AD cases and 39 controls without any significant PMD alteration between analyzed samples.

The authors found:

- KIF5A mRNA levels were significantly elevated at Braak stages III-IV and V-VI in comparison with stages I-II
- KLC1 mRNA levels were significantly elevated in late Braak stages V-VI compared to stages I-II

With respect to protein levels the authors found:

- In contrast to previous discoveries by the same group, the authors found that KIF5A protein levels did not change between Braak stages 0-II, III-IV or V-VI. It is worth mentioning that the present results were obtained working with a different cohort of control and AD brain samples than in the previous manuscripts (Hares K et al., 2017[1])
- The authors did not observe significant variations in KLC1 protein levels with Braak stages.
- No influence of SNPs within the KIF5A gene locus (rs12368653 and rs4646536) on KIF5A or KLC1 protein levels.

Correlation between Kinesin levels and amyloid precursor protein:

- As reported before, authors found that APP protein level did not differ between AD and control cases.
- The authors found that APP protein levels correlated inversely with KIF5A and KLC1 in AD
- The authors found that neither KIF5A or KLC1 correlated with the level of soluble or insoluble Aβ. The authors observed a trend, although not statistically significant, for lower levels of soluble Aβ to be associated with higher levels of KIF5A.
- The authors found that soluble and insoluble Aβ were significantly higher in AD than controls, corroborating previous reports from other groups.

Correlation between KIF5A levels and hyperphosphorylated tau:

- The levels of hyperphosphorylated tau in the soluble protein fraction was increased in AD compared with control cases, corroborating previous data from other groups.
- The levels of hyperphosphorylated tau correlated inversely with KIF5A protein level but not with KLC1 protein level in AD.
- No correlation between KIF5A or KLC1 protein levels and insoluble tau load.

With respect to SNPs in KIF5A locus linked to MS susceptibility in AD patients the authors found:

- KIF5A mRNA levels were significantly lower in AD patients homozygous for the KIF5A gene locus linked to MA susceptibility rs12368653 SNP (AA) than in AD patients with no copies (GG).
- Similarly, KIF5A mRNA expression was significantly lower in AD patients homozygous for the gene locus linked to MA susceptibility rs4646536 SNP (AA) than did heterozygous AD patients (AG).
- KLC1 mRNA expression was not significantly different in AD patients stratified according to KIF5A SNPs either rs12368653 or rs4646536

With respect to SNPs in KLC1 locus linked to AD susceptibility in AD patients the authors found:

- No significant difference in KLC1 mRNA expression between AD patients heterozygous for the rs8702 SNP (GC) and those with no copies (GG).
- No significant difference in KLC1 mRNA expression between AD patients heterozygous for the rs8007903 SNP (AG) and those with no copies (AA).
- No significant effects of KLC1 SNPs on KIF5A mRNA expression.

Discussion Section:

The authors discuss that they found KLC1 and KIF5A mRNA levels to be elevated in AD associated with Braak tangle stages. Hares and coworkers found an inverse correlation between kinesin levels and AD-associated proteins. It is worth mentioning that the inverse correlation is observed only with APP but not with soluble or insoluble Aβ, and that they observed a trend with lower levels of soluble Aβ to be associated with higher levels of KIF5A.

The authors claim that the elevated levels of KIF5A mRNA and KLC1 mRNA in AD brain samples may be a compensatory response to other pathological processes that interfere with axonal transport of protein cargoes to the synapse. The authors found lower KIF5A mRNA levels in SNPs with susceptibility for MS but no changes in protein levels; and lastly the authors found no changes in KLC1 mRNA in the same samples.
Based on all their findings the authors highlight the importance of KIF5A in maintaining axonal transport and raise the possibility that patients with higher “reserve levels” of KIF5A are less susceptible to axonal transport disruption and pathology. Similarly, the authors propose based on their results that genetic polymorphisms within KIF5A gene locus could represent a common neurodegenerative pathway across a spectrum of neurological conditions.

Personal Report:

The experimental evidences provided by Kelly Hares and co-workers, although interesting, are very preliminary and highly speculative. The authors found increased levels of mRNA for KIF5A and KLC1 in AD brain samples but did not observe any effects at protein levels. Interestingly, unlike their previous report, results obtained with a new cohort of AD and control brain samples showed that KIF5A protein levels did not changed between Braak stages 0-II, III-IV or V-VI. Similarly Hares and co-workes did not find any changes in KLC1 or KIF5A protein levels associated to different SNPs within the KIF5A gene locus (rs12368653 and rs4646536) associated with increased susceptibility to AD. Based on these results, this reviewer does not see why the authors emphatically suggest that KIF5A is highly important in maintaining axonal transport, without even analyzing other KIF5s, in particular the neuronal specific KIF5C, as well as KLC2. Recent reports have determined that functional mutations in KIF5A are associated to different progressive neuropathies, as stated by Hares and co-workers in their manuscript, highlighting the critical role that plays KIF5A in maintaining the transport of important axonal cargoes that other wise result in pathology. Similarly, it is not evident for this reviewer why the presence of more mRNA for KIF5A (termed“reserve levels”by the authors), which does not result in an increase in KIF5A protein

level, will make AD patients less susceptible to axonal transport disruption and pathology. It would be very informative if the authors could propose any particular molecular mechanism to explain such categorical statement. In summary, although preliminary in my opinion, the results obtained by Hares et al. are interesting and promising, however the authors extend too far their discoveries without proposing any particular hypothesis driven mechanism.

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

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Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I work on axonal transport and neurodegeneration.

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.