Pathological Assessment of Clusterin in Vascular and Neurodegenerative Diseases of the Brain

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Introduction

Genome wide association studies (GWAS) found significant association between the clusterin (apolipoprotein J) gene and risk for Alzheimer's disease (AD)[1]. Rapid clinical progression and severity of AD have been correlated with high levels of plasma clusterin. In the cerebral cortex of AD cases clusterin associates with Aβ40 in plaques but not Aβ42 (figure 1)[2], suggesting a mechanistic role for clusterin in amyloid clearance and a biomarker for neurodegeneration.

Clusterin has also been implicated in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), the most common type of hereditary small vessel disease (SVD) caused by mutations in the NOTCH3 gene[3]. CADASIL is characterised by granular osmiophilic material (GOM) in vessels and the degeneration of vascular smooth muscle cells (VSMCs). We have shown NOTCH3 extracellular domain (N3ECD) accumulates in GOM deposits[4], and recently other laboratories have suggested clusterin associates with GOM deposits (figure 2)[2].

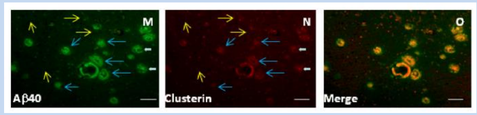

Figure 1 (above). Clusterin is co-localised with Aβ40 plaques in AD[2]. Yellow arrows : plaques positive for Aβ42 but not Aβ40 and clusterin. White arrows : plaques with Aβ40 , Aβ42 and clusterin. Blue arrows : plaques negative for Aβ42 but positive for Aβ40 and clusterin. Scale bars are 50μm.

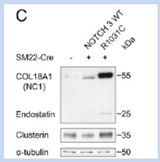

Figure 2 (left). Western blot of protein extracts taken from aortas of mice expressing WT NOTCH3 or the R1031C CADASIL mutation; clusterin is increased in aortas of mice expressing CADASIL-associated NOTCH3 mutations [3].

Aims

To investigate clusterin protein expression in post mortem brain tissue in a cohort of seven different dementing diseases, and aged matched cognitively normal control groups. Clusterin staining patterns were assessed in white matter (WM), grey matter (GM) and vessels walls in cases of vascular and neurodegenerative dementias.

	Young Controls	Old Controls	95+ Controls	SVD	CADASIL	Swedish hMID	PADMAL	DLB	CAA	AD
N (Total=69)	8	8	5	8	10	4	5	7	7	7
Mean Age (years)	57.6	85.75	99.4	83.375	58.8	42.5	50.0	75.4	83.3	76.7
(range)	(46-65)	(78-94)	(95-104)	(67-96)	(44-68)	(30-48)	(42-59)	(71-85)	(77-88)	(63-87)
Gender (M/F)	5/3	2/6	1/4	2/6	7/3	2/2	2/3	4/3	3/4	3/4
Mean Age at onset (years)				n/a	46.2	33.75	40.8	69.0	74.0	61.5
Duration of Disease				n/a	12.6	8.75	9.2	6.6	10.3	8.0

Table 1. Demographics of cases used in this cohort to analyse clusterin staining in vascular and neurodegenerative diseases.

Methods

Subjects: Three aged matched control groups to seven different dementing diseases were analysed in this study (total n=69 cases and controls, table 1).

Immunohistochemistry (IHC): 10μm thick tissue sections from the frontal lobe (Brodmann area 9) were stained with anti-clusterin antibody (Abcam Ab#69644).
Images were captured using a Zeiss Axioplan 2 microscope and analysed with Image-Pro software, measuring mean clusterin immunoreactivity (IR) and percentage area stained (%A) in the cortex (GM) and underlying white matter (WM).

WM pathological assessment: 53 tissue sections from the frontal lobe were stained with luxol fast blue and cresyl fast violet (LFB/CFV, figure 3) for assessment of WM pathology using a score between 0 (normal appearance of WM) and 3 (severe loosening of WM & severe pallor). WM pathology was scored by 2 individuals (JT and LC) and the average score used.

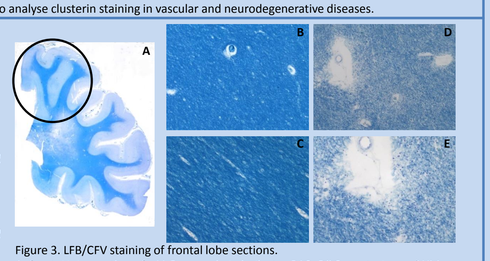

Figure 3. LFB/CFV staining of frontal lobe sections.
A: frontal lobe section of a VaD case, circle indicates BA9, B&C: control case (WM score 1), D&E: VaD case Infarct (WM score 3), B&D: 10x mag, C&E: 20x mag.

Results 1: Clusterin is associated with vascular pathology in the WM

We found no significant difference between groups for clusterin IR and %A in the GM (P>0.05; figure 4 & 5). Clusterin stained plaque-like deposits only in CAA cases, in agreement with Howlett et al[2](figure 6 middle row).

In the WM, clusterin IR was significantly higher in PADMAL (figure 4), and both CADASIL and PADMAL showed significantly greater %A (P=0.011; figure 6 top row). Clusterin stained deposits in vessel walls more frequently in WM. However, CADASIL cases had consistent vessel wall staining in both GM and WM (figure 6 bottom row). Capillary staining occurred frequently in CADASIL GM, but all groups showed capillary staining in the WM.

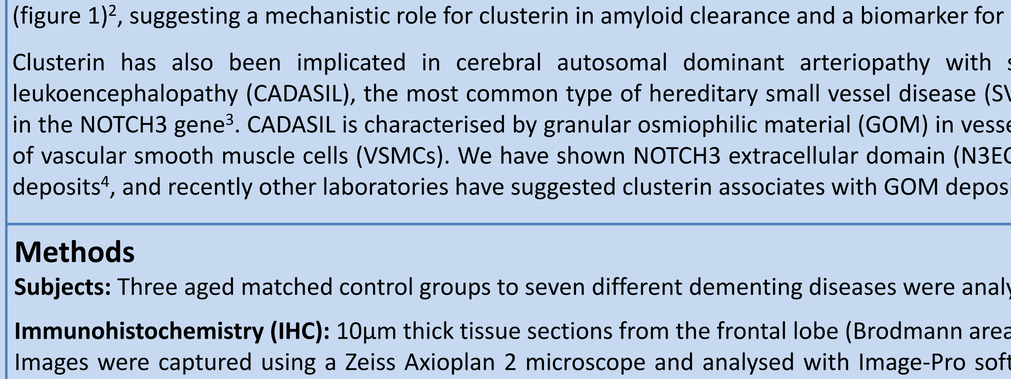

Figure 6. WM, GM and vessels labelled with clusterin by IHC . Representative images taken from each group, WM and GM at 20x mag and vessels at 40x mag.

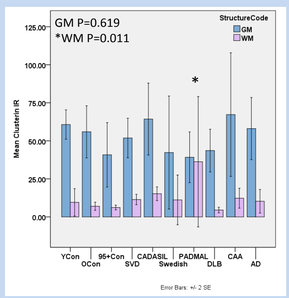

Figure 4. Mean clusterin IR in GM and WM.
No significant differences in clusterin IR in GM.
In WM clusterin IR was significantly increased in PADMAL group (Kruskal Wallis H test, P=0.011).

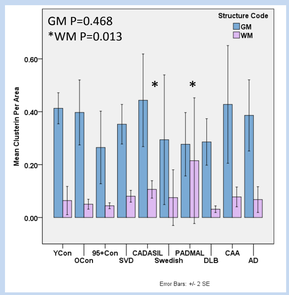

Figure 5. Clusterin percent area (%A) stain in GM and WM. No difference in %A staining in the GM. In WM, CADASIL and PADMAL groups had significantly increased %A stain (Kruskal Wallis H test, P=0.013).

Results 2: Clusterin is associated with vascular pathology and axon damage

To investigate clusterin staining in different primary disease mechanisms (hypoxic Vs neuronal dysfunction), cases were grouped into 3 categories:

1. Control: cognitively normal controls,
2. VasD: dementia of vascular origin (CADASIL, SVD, Swedish hMID, PADMAL and CAA),
3. NdD: neurodegenerative origin (AD and DLB).

Clusterin %A was not different in GM, but was significantly greater in WM of VasD group (figure 7). Mode WM score was greater in VasD (figure 8), and Spearman's rho correlation revealed a relationship between WM scores and both clusterin %A and IR, therefore indicating a relationship between clusterin and vascular based axon damage (figures 9, 10 & 11).

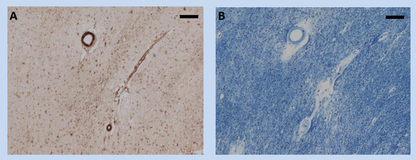

Figure 11.
Vessel and axon staining in a CADASIL case.

A: clusterin IHC

B: LFB/CFV

Bars are 100μm.

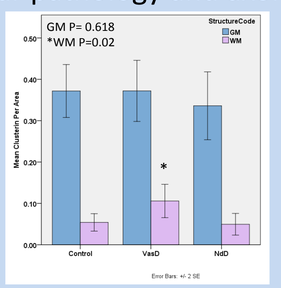

Figure 7. Mean clusterin %A in GM and WM in three groups of cases, Control, VasD and NdD. No difference in GM (P>0.05), but significant increase in %A in VasD WM (Kruskal Wallis H test, P=0.02).

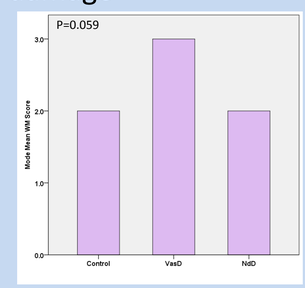

Figure 8. Mode WM score according to different primary mechanisms in control, VasD and NdD groups. There was a trend for higher WM score in VasD group (Kruskal Wallis H test, P<0.1).

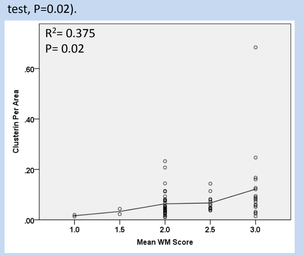

Figure 9. Relationship between clusterin %A and WM score for all 69 cases. Higher WM scores were significantly associated with greater areas of clusterin staining (Spearman's Rho, P=0.02).

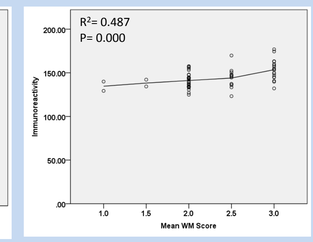

Figure 10. Relationship between clusterin IR and WM score for all 69 cases. Higher WM scores were significantly associated with greater IR of clusterin (Spearman's Rho, P=0.000).

Conclusions

Clusterin staining was found to be higher in vascular dementias and was associated with WM pathology, an indicator of axon damage frequently observed in vascular type dementias. Our work suggests clusterin may play a role in vascular type dementia, potentially through clearance of proteins at the blood brain barrier, for example Aβ40 and GOM deposits in CADASIL[2,3,5]. This indicates that clusterin in the brain is widely associated with vascular damage, and therefore the GWAS results[1] may indicate a vascular role in AD.

Acknowledgements

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References: [1] Harold et al., Nat. Genet. 2009; [2] Howlett et al., Brain Pathol. 2013; [3] Arboleda-Velasquez et al., PNAS 2011; [4] Yamamoto et al., JNEN 2013; [5] Carare et al., Neuropathol. Appl. Neurobiol. 2013.