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). 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), 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. 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, and recently other laboratories have suggested clusterin associates with GOM deposits (figure 2).

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

Immunohistochemistry (IHC): 10μm thick tissue sections from the frontal lobe (Brodman area 9) were stained with anti-clusterin antibody (Abcam Ab69664).

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.

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 (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.

Conclusions
Clusterin staining was found to be higher in vascular dementia 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. This indicates that clusterin in the brain is widely associated with vascular damage, and therefore the GWAS results may indicate a vascular role in AD.

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References

Aims
To investigate clusterin protein expression in post mortem brain tissue in a cohort of seven different demencing 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.