Microhemorrhages and small vessel disease in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)

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Introduction

CADASIL is the most common monogenic small vessel disease (SVD) and cause of hereditary stroke (1). It is caused by dominant mutations in the NOTCH3 gene that encodes for a transmembrane receptor primarily found in pericytes and vascular smooth muscle cells. NOTCH3 mutations result in accumulation of the NOTCH3 extracellular domain in arteriolar wall with subsequent medial degeneration and wall thickening, predominantly in cerebral white matter (2).

Individuals typically present with headaches, ischemic strokes and dementia over the 4th to 7th decades of life (2, 3). On magnetic resonance imaging (MRI) white matter hyperintensities are frequently seen in the external capsule and temporal pole on T2-weighted or fluid-attenuated inversion recovery (FLAIR) that can be seen decades before symptom onset (4).

CADASIL patients additionally have increased risk of cerebral microbleeds (CMBs) on MRI although CMBs are also seen with age and other SVDs such as hypertensive arteriopathy and cerebral amyloid angiopathy, albeit with differential patterns of distribution (5, 6). Despite the association of CMBs with intracerebral hemorrhage (ICH), ICHs are uncommon in CADASIL (7). Moreover, the neuropathologic correlates of CMBs are unclear (8). In this pilot study we examine microhemorrhages in four CADASIL patients in relation to other pathologic parameters of cerebrovascular disease.

Materials and Methods

Post-mortem brain tissue of four subjects from the genetically confirmed Newcastle CADASIL Cohort were examined (Table 1). Formalin fixed paraffin embedded sections from the frontal cortex and subcortical white matter, basal ganglia, and cerebellum were assessed for arteriolosclerosis (Figure 1), lacunar infarcts and microinfarcts on hematoxylin and eosin (H&E) stain based on guidelines by Skrobot et al. (9).

Vascular smooth muscle degeneration was graded on smooth muscle actin immunohistochemistry using a semi-quantitative scoring system (Table 1; Figure 2)

The distribution and degree of microhemorrhages were assessed semi-quantitatively on Prussian blue stain as 1: sparse, 2: moderate, and 3: abundant hemosiderin deposits (Figure 3).

Score	SMA staining definition
S0	no SMA staining loss
S1	partial SMA loss in vessel wall (<50%)
S2	partial SMA loss in vessel wall (>50%)
S3	complete SMA loss in vessel wall

Table 1 – SMA scoring system

Results

All four cases showed mild to severe thickening of arteriolar walls, some with characteristic basophilic staining of the tunica media (Figure 1D). (Table 2)

Degeneration of vascular smooth muscle cells was especially severe in arterioles of the frontal subcortical white matter and basal ganglia (Figure 3C-3D)

Multiple lacunar infarcts (Figure 4) or microinfarcts (Figure 5) were seen, most commonly in the subcortical white matter of the frontal lobe but also in the cortex, basal ganglia and cerebellum.

Microhemorrhages were seen in all subjects in all regions but were most frequent and pronounced in the basal ganglia around small to medium sized arterioles, although they were also seen in the brain parenchyma and around capillaries. The vessels associated with the most abundant hemosiderin deposits showed mild to moderate medial degeneration and thickening. Microhemorrhages were uncommon adjacent to infarcts.

Study no	Age	Sex	NOTCH3 mutation	Subcortical white matter				Basal ganglia			
				Infarct[1]	Arteriolosclerosis[1]	SMA score[2]	Prussian blue3	Infarct[1]	Arteriolosclerosis[1]	SMA score[2]	Prussian blue
1	44	F	R153C	Yes	3	3	1	Yes	1	2	2
2	55	M	R558C	Yes	1	0	2	Yes	1	0	3
3	68	F	R133C	No	2	0	3	Yes	1	0	3
4	68	M	R153C	Yes	2	3	2	Yes	1	0	3

Table 2: Demographic and pathologic data of select CADASIL cases from the Newcastle cohort.

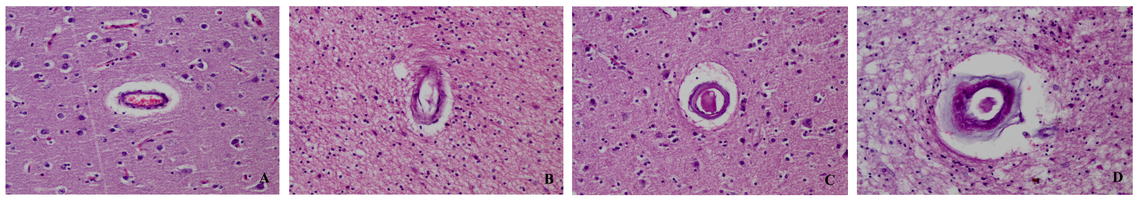

Figure 1 – H&E staining of representative sections showing varying degrees of arteriolosclerosis (A): Assigned score of 0 (B): Assigned score of 1 (C): Assigned score of 2 (D): Assigned score of 3

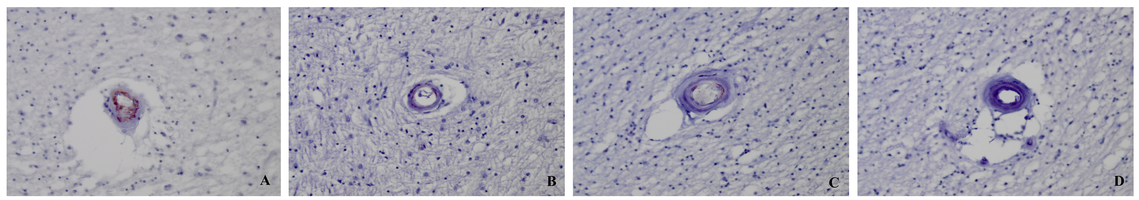

Figure 2 – Representative SMA staining of specimens (A): Showing no loss of staining and assigned an S0 score (B): Partial SMA loss in vessel wall (<50%) assigned S1 score (C): Partial SMA loss in vessel wall (>50%) assigned S2 score (D): Complete SMA loss in vessel wall assigned S3 score

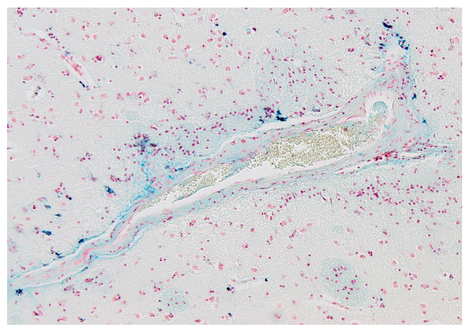

Figure 3 – Prussian blue staining showing abundant perivascular hemosiderin deposits

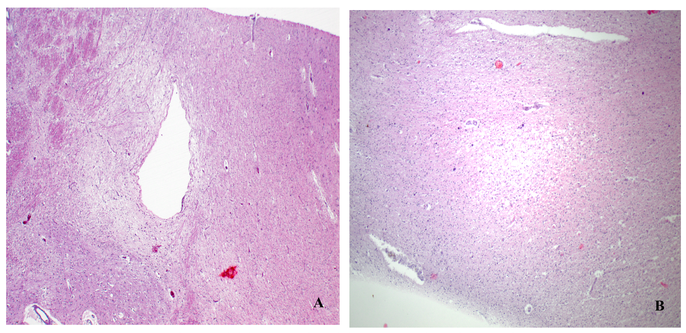

Figure 4 – H&E staining showing (A): lacunar infarct (B): intraparenchymal microinfarct

Discussion

Vascular smooth muscle degeneration was most pronounced in the subcortical white matter and basal ganglia. Microhemorrhages were common and widespread in the brains of our CADASIL patients, seen in the parenchyma and around vessels in the cortex and subcortical white matter, basal ganglia and cerebellum but most frequent and severe around arterioles in the basal ganglia (Table 2).

Microhemorrhages did not have a predilection for vessels with severe medial degeneration or near infarcts.

These preliminary results suggest that interaction of multiple factors likely underlie the pathogenesis of microhemorrhages in CADASIL.

Future studies will examine additional CADASIL patients in comparison to subjects with sporadic SVD using immunohistochemical markers for components of the neurovascular unit to better characterize and help elucidate the pathogenesis of microhemorrhages.

Our goal is to evaluate the location and prevalence of small vessel disease, microhemorrhages, and infarcts as observable by histopathology and explore the potential mechanics of such microhemorrhage. Cerebral microbleeds (CMB), the MRI correlate of the histologic microhemorrhage, are a known risk factor for intracranial hemorrhage (10). Furthermore, antiplatelet drugs, commonly used for stroke treatment and prevention, are associated with increased intracerebral hemorrhage in patients with CMB (11). For those reason, the safety of antiplatelet drugs and thrombolysis in CADASIL patient is uncertain. Our goal to evaluate the microhemorrhage's location and mechanics could potentially shine a light on this matter.

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