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# The brain lipidomes of subcortical ischemic vascular dementia and mixed dementia

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ABSTRACT

Despite its importance as the leading cause of vascular dementia, the primary pathogenic mechanisms in subcortical ischemic vascular dementia (SIVD) have remained elusive. Because of the lack of approved therapeutic agents for SIVD, there is a pressing need to identify novel therapeutic targets. Comparative lipidomic analyses of SIVD and mixed dementia (i.e., SIVD and Alzheimer's disease, MixD) may also confer new insights pertaining to the possible interaction between neurodegenerative and vascular mechanisms in the pathogenesis of dementia. Liquid chromatography coupled to mass spectrometry was used to comprehensively analyze the lipidomes of white and gray matter from the temporal cortex of nondemented controls, SIVD, and MixD subjects. Detailed molecular profiles highlighted the pathologic relevance of gray matter sphingolipid fatty acyl chain heterogeneity in dementia. In addition, the levels of sulfatides and lysobisphosphatidic acids were progressively increased in the temporal cortex gray matter from control to SIVD to MixD. White matter phospholipid profiles indicated possible adaptive mechanisms (i.e., increased unsaturation) to chronic ischemia in SIVD and elevated membrane degradation in MixD.



## 1. Introduction

Subcortical ischemic vascular dementia (SIVD) is considered a more clinically homogenous subtype of vascular dementia (VaD) and represents a leading cause of vascular cognitive impairment and dementia, arising primarily from small-artery disease and hypoperfusion (Román et al., 2002). SIVD is predominantly the result of both complete infarcts (lacunar infarcts and microinfarcts) and rarefaction in the deep cerebral white matter, also termed white matter lesions (Román et al., 2002). Although SIVD accounts substantially for cases of cognitive decline in the elderly individuals, it often remains undiagnosed (Román et al., 2002). Given the relatively long prodromal period for the development of dementia, a therapeutic window may be available for medical intervention before the onset of frank cognitive and behavioral symptoms if accurate diagnosis could be made at the preclinical or pre-dementia stage (Exalto et al., 2012; Frisoni et al., 2010). The identification of reliable markers that could delineate the early pathogenic events in dementia could therefore alleviate the escalating public health burden associated with the disease (O'Brien et al., 2003).

In the last decade, considerable progress had been achieved in understanding the Alzheimer's disease (AD), both in terms of elucidating disease pathogenesis and developing treatment strategies, which is in stark contrast to the dearth of novel data pertaining to VaD (O'Brien et al., 2003). Currently, there are no approved medications available for treating VaD, and treatment is usually confined to controlling associated vascular risk factors such as hypertension and dyslipidemia (Moretti et al., 2011). Furthermore,

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cholinesterase inhibitors and memantine designated for the treatment of AD displayed limited, if any, benefit to VaD patients (Simonsen et al., 2012). Thus, there is a pressing need to identify novel therapeutic agents effective for VaD treatment. Studies that address the role of brain lipid aberrations in VaD pathogenesis have been particularly scarce, especially in the recent decade. Perturbation of brain lipid homeostasis in VaD had been previously investigated by Wallin et al. (1989) chiefly using thin-layer chromatography. The group reported substantial reductions in cerebrosides and sulfatides, and to a smaller extent, cholesterols and phospholipids in the white matter of VaD and AD patients, respectively (Wallin et al., 1989). Nonetheless, systematic profiling of the comprehensive brain lipidome of VaD patients is still lacking and therefore requires comprehensive instrumental analyses. Indeed, molecular species within the same lipid class could be altered to varying extent or even in a reciprocal manner depending on the level of unsaturation and/or fatty acyl chain lengths, as had been previously demonstrated by Chan et al. (2012) in postmortem AD brain tissues and AD mouse models. Thus, the elucidation of a precise molecular lipid signature that defines SIVD is prerequisite for deciphering the mechanistic roles of lipids in dementia stemming from vascular-related pathologies. The clinically homogenous nature of SIVD, as well as its status as the leading cause of vascular-related dementia in the elderly individuals (Román et al., 2002), renders it a suitable candidate for investigating the role of lipids in VaD pathogenesis.

Mounting evidence indicate the frequent coexistence of brain vascular lesions and neurodegenerative changes in cognitively impaired individuals (Petrovitch et al., 2005), and it was found that these disorders were often juxtaposed in most population samples (Snowdon et al., 1997). For instance, in the Honolulu-Asia Aging Study, neuropathological examination of demented individuals revealed an appreciable overlap, as well as autonomous contributions of AD pathology and vascular infarcts to the development of dementia (Petrovitch et al., 2005). Indeed, these distinct categories of brain pathologies could possibly produce synergistic effects culminating in the clinical expression of cognitive impairment and dementia, known as mixed dementia (MixD) (O'Brien et al., 2003), defined originally by Hachinski, (1990) as "Alzheimer's disease and cerebral infarcts contributing to the dementia". Possibly, vascular damages could reduce the threshold for the clinical manifestation of AD (Esiri et al., 1999; Exalto et al., 2012). Accumulating data have shown that the occurrence of pure VaD is rare compared with the mixed phenotype (Enciu et al., 2011). Moreover, vascular risk factors including hypertension, hyperlipidemia, diabetes mellitus, and the metabolic syndrome predispose individuals to both AD and SIVD (de la Torre, 2010). Indeed, vasculopathy (and the resultant ischemia) had long been proposed as an alternative etiology of AD apart from the broadly accepted amyloid hypothesis (de la Torre, 2004; Pluta and Amek, 2008; Scheibel et al., 1989). In fact, in his seminal work in 1907, Alzheimer also noted cerebrovascular aberrations of the endothelial walls, particularly for small vessels in the brains of AD patients, which received considerably less attention compared with amyloid-beta aggregates and neurofibrillary tangles that now constitute the hallmark features of AD (Pluta and Amek, 2008). The co-occurrence of disease phenotypes implies that vascular defects and neurodegenerative changes may interact on several levels. Elucidating the molecular details of such interactions, as well as the respective contributions of vascular and Alzheimer's pathology to cognitive deficits in MixD might therefore help identify the primary pathogenic mechanism in AD per se.

In this study, we report the comprehensive lipidomic profiling of the white matter and gray matter from the temporal cortex of patients with SIVD and MixD, respectively, and in comparison to age-matched, nondemented controls using high-performance liquid-chromatography coupled to mass spectrometry (HPLC/MS) as the principal analytical platform. To our knowledge, this is the first lipidomic study to systematically characterize the distinct brain lipid molecular signatures of SIVD and MixD patients. Association between brain lipid profiles and neuropathological parameters for dementia (i.e., neuritic plaques and neurofibrillary tangles) were also investigated by comparing variations in the lipidomic patterns as a function of increasing senile plaque densities and tau pathology. This study (1) identifies novel molecular therapeutic targets for SIVD; and (2) confers new insights pertaining to the interactions between vascular and neurodegenerative lipid pathology in contributing to dementia.

## 2. Methods

### 2.1. Subjects and tissue processing

Postmortem frozen brain tissues from SIVD, MixD, and age-matched normal control subjects (Supplementary Table S1) were obtained from the Newcastle Brain Tissue Resource, Institute for Ageing and Health, Newcastle University. Informed consent was obtained from the guardians of the patients before donation of brain tissues and approval was granted by local research ethics committees (National University Health System, Singapore and Newcastle upon Tyne Hospitals Trust, UK). For this study, we assessed samples of both gray and white matter from the temporal lobe (Brodmann area 21). We focused on the temporal lobe because medial temporal lobe atrophy is a common finding in dementia and our recent study suggested a vascular basis for neurodegeneration (Gemmell et al., 2012). The temporal lobe is also relatively free of large infarcts (Kalaria et al., 2004).

Final classification of subjects was assigned based on established neuropathological diagnostic criteria. Briefly, hematoxylin–eosin staining was used for assessment of structural integrity and infarcts, Nissl and Luxol fast blue staining for cellular pattern and myelin loss, Bielschowsky silver impregnation for "CERAD" rating of neuritic plaques (Mirra et al., 1991) and tau immunohistochemistry for "Braak" staging of neurofibrillary tangles (Braak and Braak, 1991). A diagnosis of SIVD was made when there were multiple or cystic infarcts, lacunae, microinfarcts, and small vessel disease, and Braak stage $<$ III (Kalaria et al., 2004). The diagnosis of MixD was assigned when there was evidence of significant AD pathology, namely Braak stage V-VI, and moderate-severe vascular pathology. Vascular pathology was graded according to the scoring system described previously (Deramecourt et al., 2012). Control subjects had no clinical evidence of dementia, neurologic, or psychiatric disease. The age-matched controls had no history of cognitive or psychiatric symptoms and died from nonneurologic causes such as bronchopneumonia, pulmonary embolism, and cardiac failure. At postmortem, control brains were Braak stage III or below and did not meet diagnostic criteria for AD (Mirra et al., 1991) or SIVD (Kalaria et al., 2004). Furthermore, tissue samples from controls were determined not to have sufficient pathology to reach the threshold to ascertain a diagnosis for dementia.

### 2.2. Lipid extraction

Frozen tissues were inactivated with 900 μL of chloroform:methanol (1:2) containing 10% deionized $H_2O$. Tissue samples were cut into fine pieces using micro-scissors on dry ice, and methanol was used to thoroughly wash the micro-scissors between samples. Samples were homogenized and incubated at 1200 rpm, 4 °C, for 1 hour in a thermomixer. At the end of the incubation, 400 μL of deionized $H_2O$ and 300 μL of chloroform were added and vortexed. Samples were then centrifuged at 12000 rpm, 4 °C, for