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Author/Contributor:
Wong, Jenny; Garner, Brett; Halliday, Glenda; Sharpe, Laura J; Brown, Andrew J

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Is Seladin-1 really a Selective Alzheimer's Disease Indicator?

Laura J. Sharpe† [a], Jenny Wong† [b], Brett Garner [b], Glenda M. Halliday[c], and Andrew J. Brown* [a]

[a] School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia

[b] Illawarra Health and Medical Research Institute and the School of Biological Sciences, University of Wollongong, Wollongong, NSW, 2522, Australia

[c] Neuroscience Research Australia and University of New South Wales, Sydney, NSW, 2031, Australia

Running title: Seladin-1 and Alzheimer's Disease

† These authors contributed equally to this work.

* To whom correspondence should be addressed:

Andrew J. Brown, BABS, School of Biotechnology and Biomolecular Sciences, Biological Sciences Building D26, University of New South Wales, Sydney, NSW 2052, Australia.

Phone: +61-2-9385-2005;

Fax: +61-2-9385-1483;

E-mail: aj.brown@unsw.edu.au

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Abstract

Selective Alzheimer's Disease Indicator-1 (Seladin-1) was originally identified by its down-regulation in the brains of Alzheimer's Disease (AD) patients. Here, we re-examine existing data and present new gene expression data that refutes its role as a selective AD indicator. Furthermore, we caution against the use of the name "Seladin-1" and instead recommend adoption of the approved nomenclature, 3â-hydroxysterol Ä^{24}-reductase (or DHCR24), which describes its catalytic function in cholesterol synthesis. Further work is required to determine what link, if any, exists between DHCR24 and AD.

Literature often refers to *Seladin-1* as being down-regulated in affected brain regions of Alzheimer's Disease (AD) patients. The acronym, Selective Alzheimer's Disease Indicator-1, is a nomenclature that encourages its reputation for being differentially expressed in AD. Peri and Serio [1] suggested that "Seladin-1" may be inappropriate considering its known roles now extend far beyond the apparent down-regulation observed in AD. We critically evaluate the evidence that Seladin-1 is a selective AD indicator. This is essential considering that AD treatments may be based on the reported down-regulation of Seladin-1 (e.g. [2, 3]).

Seladin-1 was identified in 2000 by Greeve *et al.* as a gene with differing expression levels between regions of AD brains but no difference in control brains [4]. Northern blotting showed that in three AD brains, Seladin-1 RNA levels were lower in temporal than frontal cortex. Seladin-1 protein levels reflected this pattern in two AD brains. Their single control brain showed equal Seladin-1 RNA levels in both temporal and frontal cortex. Whilst frequently cited as establishing Seladin-1 as a selective AD indicator, these findings must be reproduced by independent groups using multiple independent cohorts of sufficient sample size, with state-of-the-art methodologies. In Greeve *et al.* [4], it is critical to note that a very limited sample size was investigated, and that the techniques used (e.g. Northern blotting) have since been surpassed by more accurate and quantitative methods.

Iivonen *et al.* subsequently examined Seladin-1 mRNA levels in the temporal vs. occipital cortex of AD brains by semi-quantitative RT-PCR [5]. Using a larger sample size, they found only seven out of 13 AD brains had lower Seladin-1 mRNA levels in temporal compared to occipital cortex, whereas their six non-AD brains had no difference or higher expression. As such, this data does not support the contention that Seladin-1 is a selective indicator of AD. However, the decrease in Seladin-1 gene expression was significant when

considering the specific AD hallmarks of neurofibrillary tangles (NFTs) and neuritic plaques, but not when comparing those without such lesions, or with other markers such as α-synuclein or amyloid-β (Aβ) pathologies. Additionally, Seladin-1 polymorphisms are associated with AD in some [6, 7], but not all studies [8].

By contrast, larger-scale, microarray studies failed to identify Seladin-1 as differentially regulated in AD. Blalock *et al*. [9] examined gene expression in hippocampi from 22 AD brains, and nine controls. Using microarray analysis and correlating gene expression with known AD markers, including NFTs, they found thousands of genes differentially regulated across the AD hippocampus. When comparing only control and early stage AD brains, they still identified several hundred differentially regulated genes. However, Seladin-1 was not among these (Figure 1A). In a recent follow-up study, Blalock *et al.* [10] improved upon their initial microarray study [9] by selectively isolating grey matter from the same brain samples using laser capture microdissection (LCM). This confirmed their initial findings that Seladin-1 expression was not significantly different in AD [10].

In another microarray study, also using LCM, Dunckley *et al*. [11] selectively isolated neurons from regions with or without NFTs from the entorhinal cortex of 19 AD brains and 14 controls. Of the 225 genes consistently up- or down-regulated, Seladin-1 was not among them [Figure 1B (NFT vs. non-NFT)], though our calculations suggest borderline significance (t = 3.254, df = 8, p = 0.012, by t-test, whereas the authors used a more stringent significance cut-off of $p < 0.01$). In a follow-up study by the same group [12], Seladin-1 mRNA expression was confirmed not to change in pyramidal neurons isolated from the entorhinal cortex. However, Seladin-1 expression was down-regulated in AD in the hippocampus and medial temporal and posterior cingulate cortices.

Both Liang *et al.* [12] and Blalock *et al.* [10] utilized LCM to isolate brain tissue for subsequent microarray analyses and the same gene chip (Affymetrix Human Genome U133 Plus 2.0), but the selected regions differed, perhaps accounting for the contrasting findings. Moreover, although LCM allows for selective and targeted isolation of cells from a region of interest, stringent RNase-free conditions during tissue handling are required as mRNAs are rapidly degraded by ubiquitous RNases and are sensitive to fixation protocols. In Liang *et al.* [12], tissue sections were fixed and stained prior to LCM; moreover, no data was presented regarding the RNA quality and integrity.

To investigate the putative Seladin-1/AD link, we used quantitative 'real-time' polymerase chain reaction (qRT-PCR) to determine Seladin-1 expression in control vs. AD brains from four brain regions. Brain tissues from the hippocampus and cerebellum (6 AD, 5 age-range and gender matched controls, [13, 14]), and the temporal and occipital cortices (9 AD, 8 age-range, gender and post-mortem interval matched controls) were all from cases longitudinally evaluated to autopsy. We used total RNA isolated from fresh frozen brain tissue from each brain region for cDNA synthesis and gene expression analyses as this yields higher quality RNA and better recovery of low abundance transcripts. In addition, we used primers that target the coding region of Seladin-1 to circumvent the 3' bias that is inherent in gene expression profiling by microarray.

Seladin-1 expression was normalized using the geometric mean of three stable, low variability housekeeping genes of high, medium or low expression as this is more effective than one single housekeeping gene in removing non-specific variation in a given sample to reveal true gene expression differences [15]. We found no difference in Seladin-1 gene expression levels between control and AD brains in any of the four brain regions examined

(Figure 2A, B). Moreover, in a paired comparison between less and more affected brain regions within the same AD cases, as in the seminal studies by Greeve *et al.* [4] and Iivonen *et al.* [5], Seladin-1 gene expression was not altered in more affected (hippocampus, temporal cortex) versus less affected (cerebellum, occipital cortex) brain regions (Figure 2C, D).

Although Seladin-1 may not necessarily be down-regulated in AD, it may still play a neuroprotective role, in which case treatments that upregulate Seladin-1 may be beneficial for AD. In the original Seladin-1 report [4], overexpression of Seladin-1 protected cells from Aβ toxicity and cell death through inhibition of caspase-3 activity. Silencing Seladin-1 using siRNA increased caspase-3 activity and ultimately Aβ production [16].

Seladin-1 has been further characterized in the last decade and identified as the ultimate enzyme in cholesterol synthesis – 3β-hydroxysterol Δ^{24}-reductase (a.k.a 24-dehydrocholesterol reductase, or DHCR24, EC: 1.3.1.72), catalysing the conversion of desmosterol to cholesterol [17]. Using a mouse model of AD (APPSLxPS1mut), Vanmierlo *et al*. [18] found that desmosterol levels were increased in AD mice, which was accompanied by a decrease in Seladin-1 mRNA. However, as Seladin-1 was upregulated at 9 months and down-regulated at 21 months, this may be secondary to AD pathology rather than causative. Accordingly, Seladin-1 expression was reduced in both cortex and cerebellum [18], but Aβ deposits occur in cortex and not cerebellum [19], again suggesting a secondary rather than causative association.

In a Seladin-1 knockout mouse model, Crameri *et al*. [20] found reduced brain cholesterol levels, and increased amyloid-β protein precursor (AβPP) processing and Aβ accumulation. These observations were reversed when Seladin-1 was overexpressed in SH-

SY5Y human neuroblastoma cells, again implicating a neuroprotective role for Seladin-1. Whilst an association between lower Seladin-1 expression levels and AD markers in a knockout mouse model is informative, it does not directly address the issue of whether Seladin-1 gene expression levels are lowered in human AD brains.

The finding that reduced cholesterol levels leads to increased AβPP processing and Aβ accumulation has also been observed by others (e.g. [21]), and AD patients may have lowered brain cholesterol levels [22]. A lowering of cholesterol levels would be expected if Seladin-1 is decreased; however increased cholesterol levels may also increase AD risk (e.g. [23]). Clearly, the relationship between cholesterol and AD is controversial, and requires further investigation (reviewed in [24]).

Given the possible link between cholesterol and AD, it is not surprising that statins, which inhibit cholesterol synthesis, have been proposed as a potential treatment [3, 25]. Additionally, statin use has been associated with a decreased risk of AD [25]. However, there are caveats to consider (reviewed in [26]). For example, it is likely that only lipophilic statins are able to cross the blood brain barrier and decrease cholesterol synthesis [27], but the decreased risk of AD was not dependent on this ability [25]. Furthermore, non-statin cholesterol-lowering drugs do not have the same effect, suggesting that lowering of cholesterol levels itself may not influence AD risk [25].

Whilst Seladin-1 was originally identified as being down-regulated in some AD brains, this name is a misnomer as it implies that Seladin-1 plays an important role in AD based on ambiguous data. Moreover, there are several other genes (e.g. ApoE, AβPP, Presenilin-1 and -2) that are far better correlated with AD. Therefore, we urge caution when claiming that Seladin-1 is down-regulated in AD, and suggest that the name DHCR24 should be used

for this gene. Further work is required to determine what link, if any, exists between DHCR24 and AD as the possibility remains that DHCR24 is involved in a subgroup of AD patients, which may help explain the original findings of Greeve *et al.*[4].

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Figure legends

Figure 1

Seladin-1 is not consistently down-regulated in Alzheimer's Disease (microarray)

Seladin-1 microarray expression data from **A)** 31 hippocampus samples including early stage (incipient), moderate, and severe AD (■) vs. controls (●) [9] and **B)** 9 entorhinal cortex samples with (■) or without (●) neurofibrillary tangles (NFTs) from the same AD brain [11], as extracted from the National Center for Biotechnology Information's Geo Profiles Database, October, 2011. ◇ indicates mean, which has been set to 1 for non-NFT.

Figure 2

Seladin-1 is not down-regulated in Alzheimer's Disease (qRT-PCR)

Seladin-1 expression was determined by qRT-PCR using RNA from **A) and B)** control (●) and severe AD (■) brains in **A)** cerebellum (5 controls, 6 AD) and hippocampus (5 controls, 6 AD) and **B)** occipital cortex (7 controls, 9 AD) and temporal cortex (8 controls, 8 AD). Data were normalised to the geometric mean of three housekeeping genes (porphobilinogen deaminase, â-actin, and peptidylprolyl isomerase A), and the control was set to a mean of 1 for each brain region. Outliers were removed. Control vs. AD: cerebellum: t = 0.15, df = 10, p = 0.88; hippocampus: t = -0.13, df = 10, p = 0.90; occipital cortex: t = -0.70, df = 14, p = 0.49; temporal cortex: t = 0.34, df = 14, p = 0.74) **C) and D)** Paired comparison for AD cases for **C)** cerebellum (●) and hippocampus (■) and **D)** occipital cortex (●) and temporal cortex (■). ◇ indicates mean, which has been set to 1 for cerebellum in C) and occipital cortex in D).

Figure 1

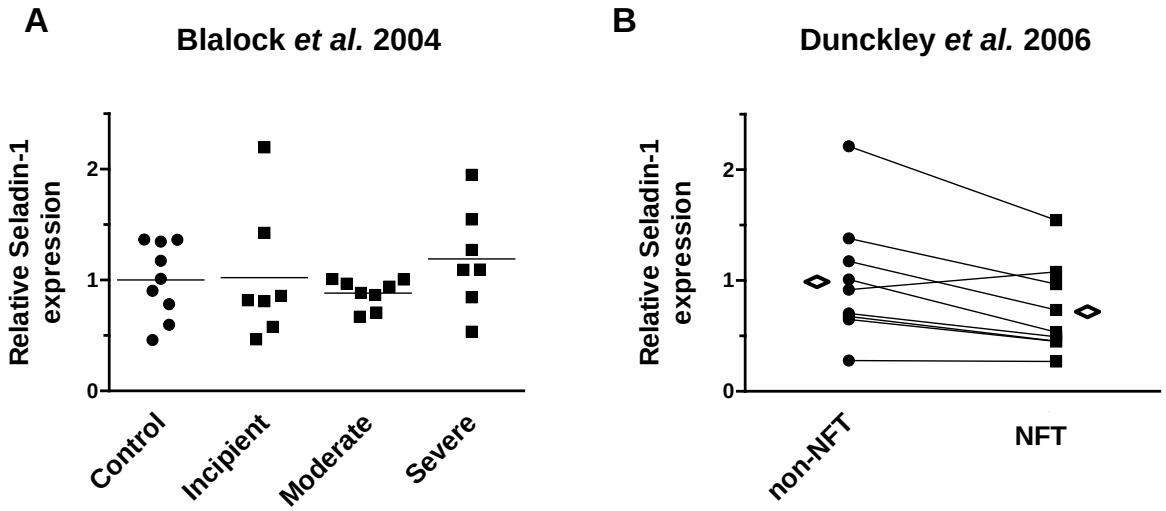
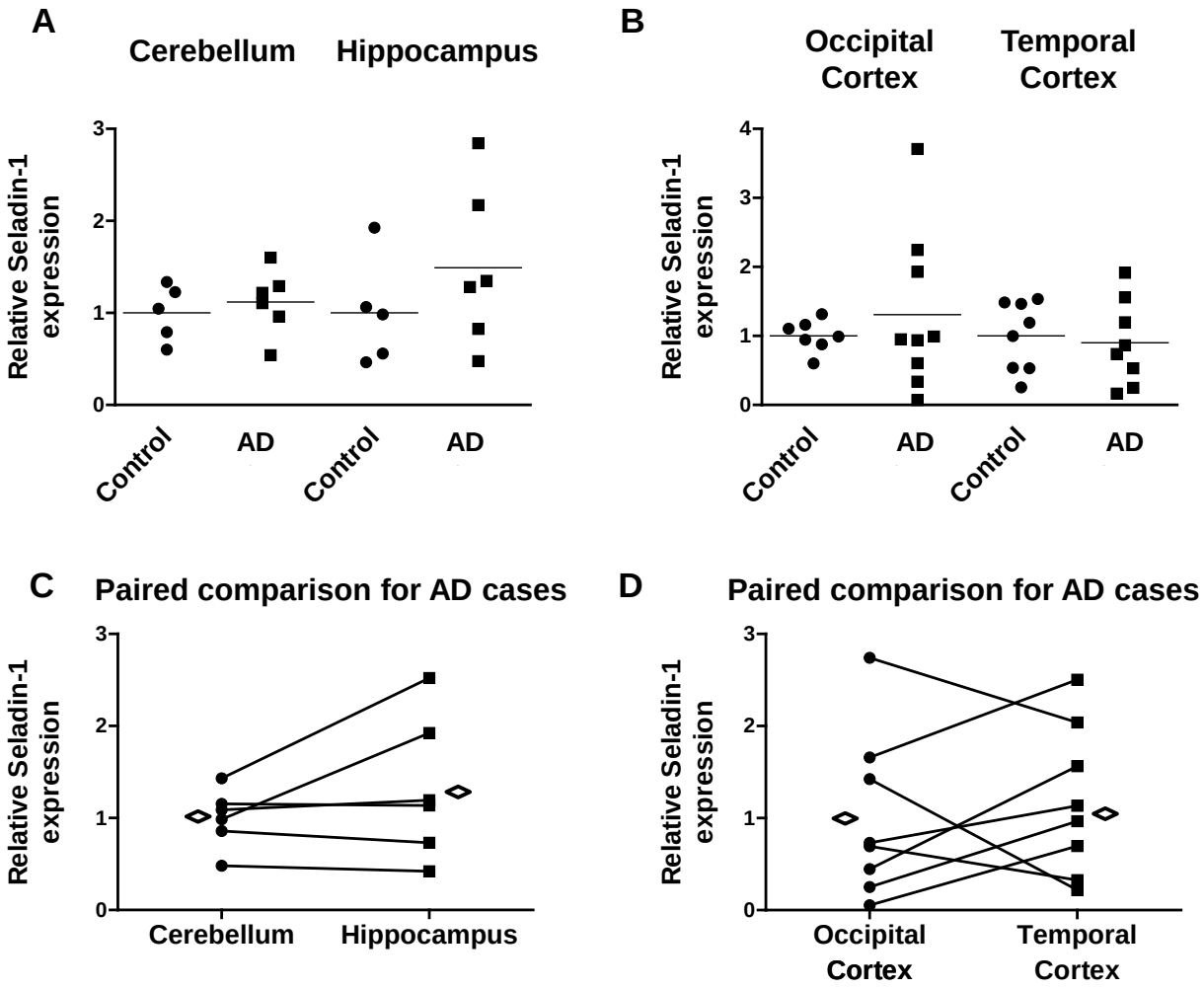

A
Blalock *et al.* 2004
Relative Seladin-1 expression
2
1
0
Control
Incipient
Moderate
Severe
B
Dunckley *et al.* 2006
Relative Seladin-1 expression
2
1
0
non-NFT
NFT

Figure 2


A
Cerebellum
Hippocampus
Relative Seladin-1 expression
Control
AD
Control
AD
B
Occipital Cortex
Temporal Cortex
Relative Seladin-1 expression
Control
AD
Control
AD
C
Paired comparison for AD cases
Relative Seladin-1 expression
Cerebellum
Hippocampus
D
Paired comparison for AD cases
Relative Seladin-1 expression
Occipital Cortex
Temporal Cortex