Author/s:
Dean, B;Duce, J;Li, Q-X;Masters, CL;Scarr, E

Title:
Lower levels of soluble β-amyloid precursor protein, but not β-amyloid, in the frontal cortex in schizophrenia

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Highlights

- There are lower levels of soluble amyloid precursor protein in Brodmann's area (BA) 6 from people with schizophrenia.
- The lower levels of amyloid precursor protein in BA 6 are not restricted to one of two defined sub-groups within the syndrome of schizophrenia.
- We show that levels of levels of Aβ1-40 or Aβ1-42 are not changed in Brodmann's area 6 from people with schizophrenia.
- Our data in schizophrenia does do not reflect the changes in these markers in the CNS of people with Alzheimer's disease.

Abstract

We identified a sub-group (25%) of people with schizophrenia (muscarinic receptor deficit schizophrenia (MRDS)) that are characterised because of markedly lower levels of cortical muscarinic M1 receptors (CHRM1) compared to most people with the disorder (non-MRDS). Notably, bioinformatic analyses of our cortical gene expression data shows a disturbance in the homeostasis of a biochemical pathway that regulates levels of CHRM1. A step in this pathway is the processing of amyloid precursor protein (APP) and therefore we postulated there would be altered levels of APP in the frontal cortex from people with MRDS. Here we measure levels of CHRM1 using [^{3}H]pirenzepine binding, soluble APP using Western blotting and Aβ1-40 and Aβ1-42 using ELISA in the frontal cortex (Brodmann's area 6: BA 6; MRDS = 14, non-MRDS = 14, controls = 14). We confirmed the MRDS cohort in this study had the expected low levels of [^{3}H]pirenzepine binding. In addition, we showed that people with schizophrenia, independent of their sub-group status, had lower levels of sAPP compared to controls but did not have altered levels of Aβ1-40 or Aβ1-42. In conclusion, whilst changes in sAPP are not restricted to MRDS our data could indicate a role of APP, which is important in axonal and synaptic pruning, in the molecular pathology of the syndrome of schizophrenia.

Figure (300 dpi and editable format)

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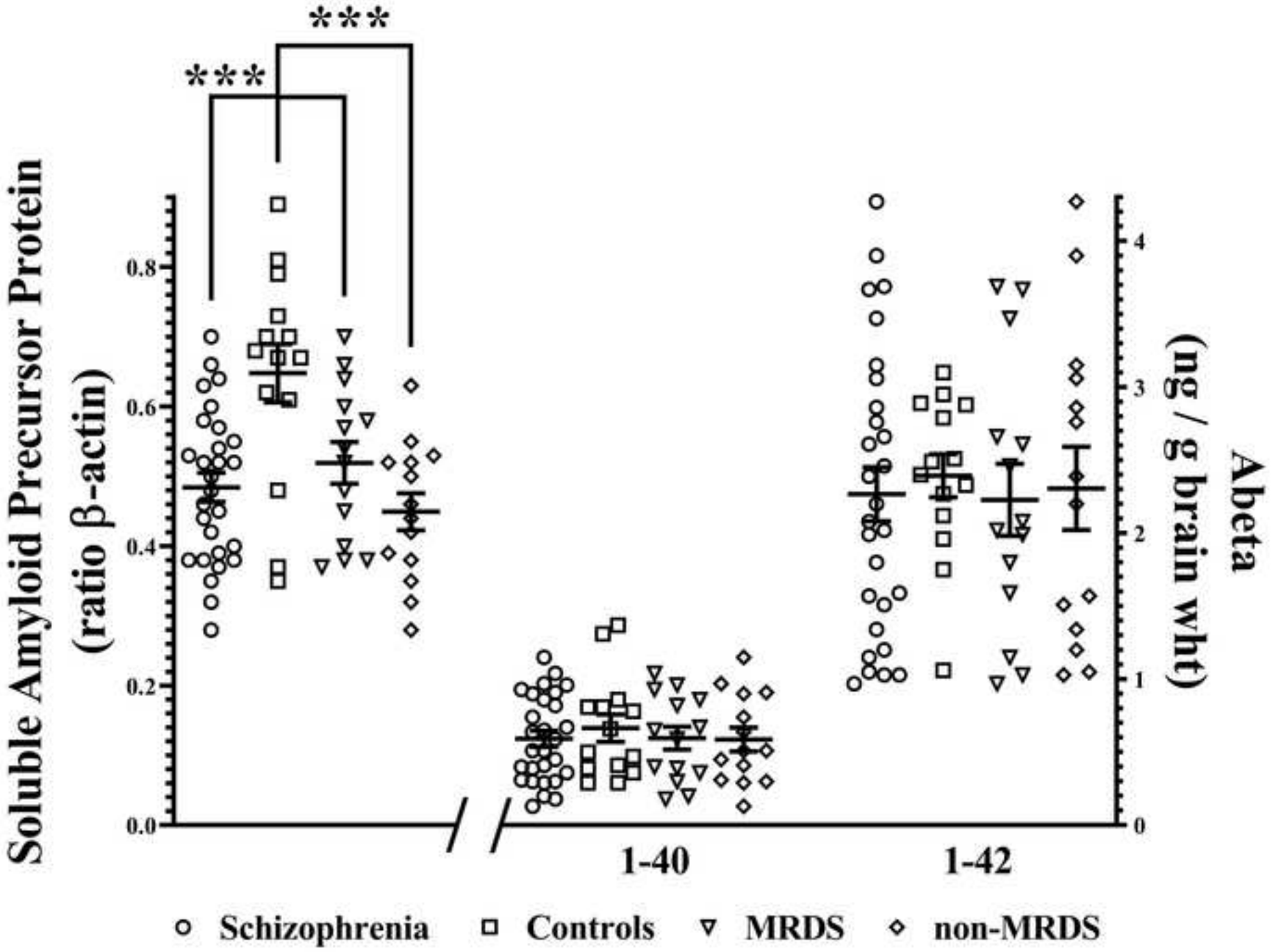

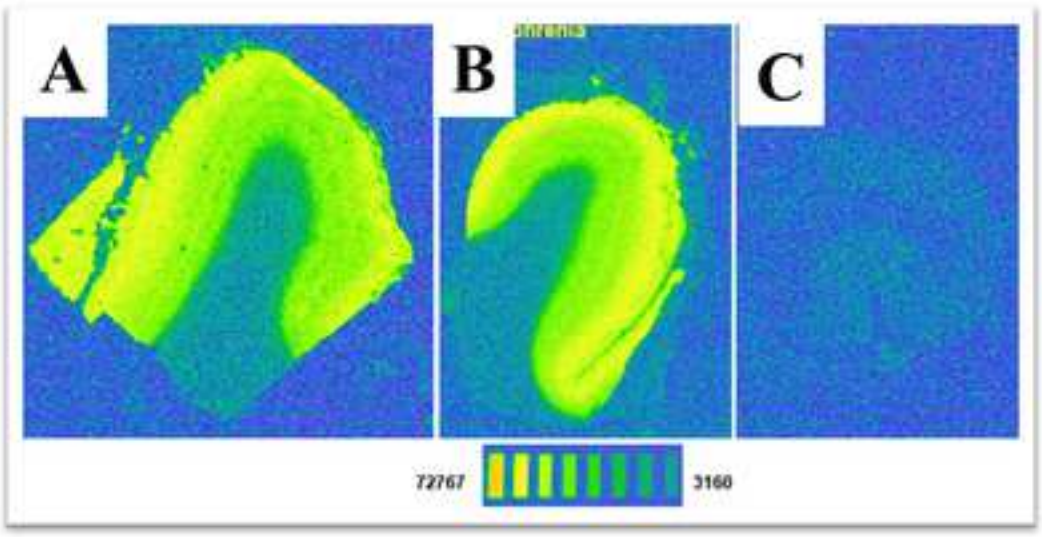

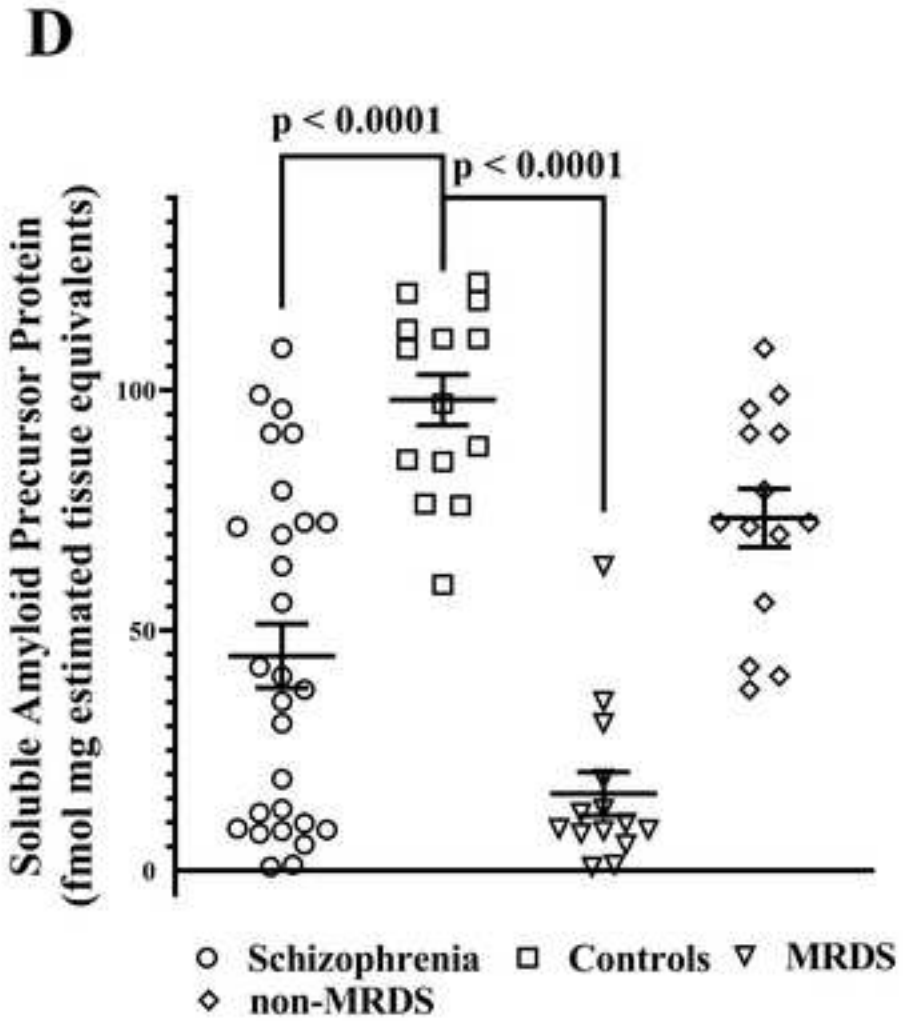

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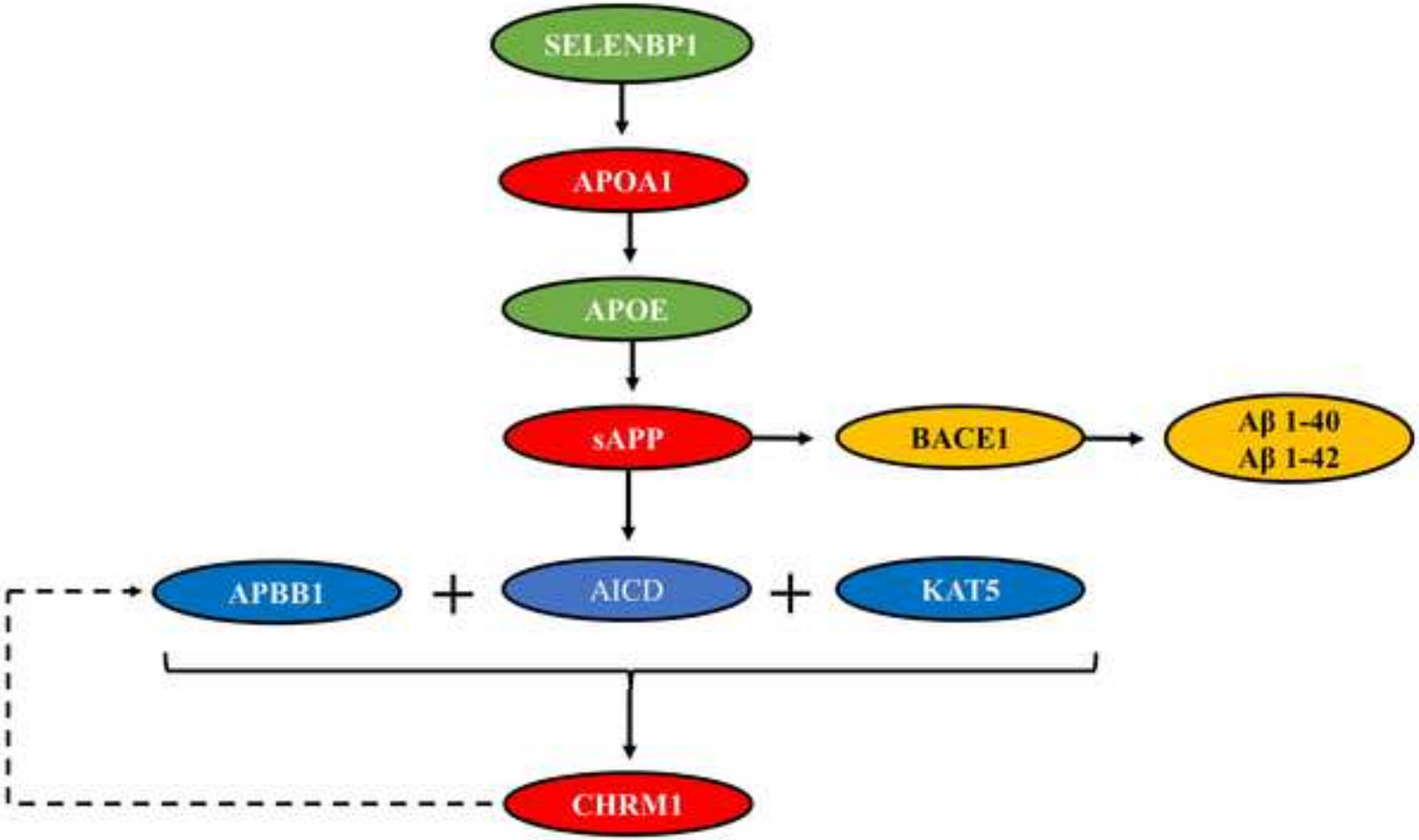

Table 1: Summary of demographic, CNS collection, treatment, symptom rating and experimental data from the cases used in this study.

	Cont.	Schizo.	p	MRDS	Non-MRDS	F: d.f.	p	MRDS vs Cont	Non-MRDS vs Cont	MRDS Vs non-MRDS
n	14	28		14	14					
Age (mean ± SEM, yr)	44 ± 5.1	47 ± 3.5	0.58	45 ± 5.0	49 ± 5.0	0.29: 2, 39	0.75			
Sex (Male / Female)	12 / 2	24 / 4	> 0.999	12 / 2	12 / 2		> 0.999			
CNS pH	6.33 ± 0.06	6.20 ± 0.04	0.08	6.26 ± 0.04	6.13 ± 0.08	2.8: 2, 39	0.07			
PMI (mean ± SEM, min)	42 ± 3.9	42 ± 2.1	0.90	42 ± 2.5	44 ± 3.4	0.16: 2, 39	0.86			
Brain Wht (g)	1377 ± 38	1441 ± 29	0.20	1479 ± 37	1393 ± 43	2.1: 2, 35	0.14			
Suicide completion (Yes / No)	0 / 14	9 / 19	0.02	6 / 8	3 / 11		0.02	0.02	0.22	0.42
DI (yr)		20 ± 3.1		19 ± 4.5	21 ± 4.3		0.76			
FRADD (mean ± SEM, fmol / mg chor. Eq.)		535 ± 129		483 ± 155	600 ± 226		0.66			
[^{3}H]pirenzepine binding (mean ± SEM, fmol / mg ETE)	98 ± 5.2	45 ± 6.6	< 0.0001	16 ± 4.5	73 ± 6.1	63: 2, 39	<0.0001	<0.0001	0.051	<0.0001
Amyloid Precursor Protein (ratio β-actin)	0.65 ± 0.04	0.48 ± 0.02	0.003	0.52 ± 0.03	0.45 ± 0.03	9.2: 2, 39	0.0005	0.02	0.0004	0.31
Aβ1-40 (mean ± SEM, ng / g brain wht)	0.67 ± 0.09	0.59 ± 0.06	0.48	0.60 ± 0.08	0.58 ± 0.08	0.25: 2, 39	0.78			
Aβ1-42 (mean ± SEM, ng / g brain wht)	2.39 ± 0.15	2.37 ± 0.18	0.66	2.23 ± 0.25	2.31 ± 0.28	0.12: 2, 39	0.88			
Aβ1-42/Aβ1-40 ratio (mean ± SEM)	4.35 ± 0.53	4.96 ± 0.63	0.53	4.91 ± 0.86	5.00 ± 0.95	0.20: 2, 39	0.82			

Abbreviations: AUC = area under the curve, Cont. = Controls, d.f. = degrees of freedom, MRDS = sub-group with schizophrenia and low radioligand binding, non-MRDS = sub-group with schizophrenia but not low levels of radioligand binding, Schizo. = Schizophrenia, SEM = Standard error of the mean

Declaration of Interest

The authors have no conflicts of interest to declare.

Contributions

JD, ES and BD conceptualised the study. JD and QL generated the data on sAPP and Aβ respectively. BD completed all statistical analyses and produced the first draft of the manuscript. All authors contributed to the final version of the manuscript submitted for publication.

Lower Levels of Soluble β-Amyloid Precursor Protein, but not β-amyloid, in the Frontal Cortex in Schizophrenia.

Brian Dean[a,b,*], James Duce[c], Qiao-Xin Li[a,b], Colin L. Masters[a,b] and Elizabeth Scarr[d].

a. The Florey, Parkville, Victoria, Australia.
b. The University of Melbourne of Melbourne Florey Department of Neuroscience and Mental Health, Parkville, Victoria, Australia.
c. MSD Discovery Centre, 120 Moorgate, London, London, UK.
d. The Department of Psychiatry, the University of Melbourne, Parkville, Victoria, Australia.

[*] Corresponding Author: Professor Brian Dean, The Synaptic Biology and Cognition Laboratory, The Florey, 30 Royal Parade, Parkville, Victoria 3052, Australia.

email: brian.dean@florey.edu.au

Abstract

We identified a sub-group (25%) of people with schizophrenia (muscarinic receptor deficit schizophrenia (MRDS)) that are characterised because of markedly lower levels of cortical muscarinic M1 receptors (CHRM1) compared to most people with the disorder (non-MRDS). Notably, bioinformatic analyses of our cortical gene expression data shows a disturbance in the homeostasis of a biochemical pathway that regulates levels of CHRM1. A step in this pathway is the processing of β-amyloid precursor protein (APP) and therefore we postulated there would be altered levels of APP in the frontal cortex from people with MRDS. Here we measure levels of CHRM1 using [^{3}H]pirenzepine binding, soluble APP (sAPP) using Western blotting and amyloid beta peptides (Aβ1-40 and Aβ1-42) using ELISA in the frontal cortex (Brodmann's area 6: BA 6; MRDS = 14, non-MRDS = 14, controls = 14). We confirmed the MRDS cohort in this study had the expected low levels of [^{3}H]pirenzepine binding. In addition, we showed that people with schizophrenia, independent of their sub-group status, had lower levels of sAPP compared to controls but did not have altered levels of Aβ1-40 or Aβ1-42. In conclusion, whilst changes in sAPP are not restricted to MRDS our data could indicate a role of APP, which is important in axonal and synaptic pruning, in the molecular pathology of the syndrome of schizophrenia.

Keywords: Amyloid precursor protein, muscarinic M1 receptor, frontal cortex, postmortem, schizophrenia, Abeta

1.1 Introduction

Data from many lines of research suggest that the muscarinic M1 and M4 receptors (CHRM1 and 4) are important in the molecular pathogenesis and treatment of schizophrenia (Dean and Scarr, 2020; Paul et al., 2022). We were the first to report that, compared to controls, levels of [^{3}H]pirenzepine binding were lower in the striatum (Dean et al., 1996), dorsolateral prefrontal cortex (Crook et al., 2001) and hippocampus (Crook et al., 2000) from people with schizophrenia. Importantly, we have used human cloned CHRMs and tissue from Chrm knockout mice to show that, using our methodology, [^{3}H]pirenzepine has a ≥ 85 % selectivity for the CHRM1 (Scarr and Dean, 2008). These data, coupled with the finding that the CHRM1 is the most abundant CHRM in the cortex (Flynn et al., 1995), means the lower levels of [^{3}H]pirenzepine binding we have reported in many areas of the cortex in people with schizophrenia are reflecting lower levels of CHRM1 (Gibbons et al., 2013). This argument is supported by Western blot data showing lower levels of CHRM1 (Dean et al., 2002), but not CHRM2, CHRM3 (Scarr et al., 2006) or CHRM4 (Dean et al., 2002) in the frontal cortex of people with the disorder.

It is now widely accepted that progress towards fully understanding the molecular pathogenesis of the syndrome of schizophrenia will require the study of intermediate phenotypes defined using biological criteria (Tamminga and Holcomb, 2005). We have been leaders in the use of intermediate endophenotypes because we study the molecular pathology of an intermediate phenotype within schizophrenia defined by [^{3}H]pirenzepine binding being ≤ 110 fmol / mg estimate tissue equivalents (ETE) in Brodmann's area (BA) 9) (Dean et al., 2023; Scarr et al., 2009). This endophenotype, termed– the muscarinic receptor deficit sub-group (MRDS), contains 25% of people diagnosed as having schizophrenia and, compared to non-psychiatric

controls, has a 98.6% specificity and a 91.3% sensitivity for people with schizophrenia with levels of [^{3}H]pirenzepine binding ≤ 110 fmol / mg ETE. Significantly, we have recently reproduced our finding of an intermediate endophenotype within schizophrenia based on [^{3}H]pirenzepine binding using tissue from another brain bank and, because of the availability of additional clinical information, we were able to show those with MRDS were less cognitively impaired than those in the non-MRDS sub-group (Dean et al., 2023). We postulated this lower level of cognitive impairment in MRDS was because they have higher levels of α7 nicotinic receptors, which would be a pro-cognitive driver (Olincy et al., 2006). Being able to propose an interactive cholinergic receptor hypothesis as the reason for less severe cognitive deficits in MRDS shows the benefit of studying intermediate endophenotypes in schizophrenia.

In our studies on the molecular pathology of schizophrenia we compared levels of coding and non-coding RNA in BA9 from people within the MRDS and non-MRDS groups to that in non-psychiatric controls (Scarr et al., 2018). A bioinformatic analysis of these different changes in gene expression suggested a complex biochemical interactome was acting to decrease levels of the CHRM1 in people with MRDS. A limitation of this analyses was that it was based on changes at the level of RNA and, due to the multiple controls on gene translation (Gibbons et al., 2018; Nelson and Cox, 2005), did not necessarily reflect functional changes at the level of the proteome. However, this limitation was lessoned because some of the components of this interactome, such as selenium binding protein (Chau et al., 2018), apolipoprotein A1 (Huang et al., 2008; Martins-De-Souza et al., 2010) and apolipoprotein E (Dean et al., 2003; Digney et al., 2005; Martins-De-Souza et al., 2010), have been shown to be altered at the level of protein in CNS or blood from people with schizophrenia.

The fourth component of a pathway defined by our interactome was ~~A~~β-amyloid precursor protein (APP) which has been implicated in the molecular pathogenesis of Alzheimer's disease (AD),~~-~~ another disorder that has a cholinergic deficit (Delport and Hewer, 2022). Moreover, APP has important roles in neurogenesis, neurite outgrowth, axon guidance and synaptogenesis (Chau et al., 2023), all of which have also been argued to be important in the pathophysiology of schizophrenia (Goo et al., 2023; Habela et al., 2016; Räsänen et al., 2022; Sheu et al., 2019). In addition, lower levels of APP have been reported in cerebrospinal fluid (CSF) (Hidese et al., 2020) and platelets (Tereshkina et al., 2020) from people with schizophrenia. Surprisingly, we are not aware of any studies of APP in the CNS from people with schizophrenia. This is a significant gap in the literature as almost all the proteins normally present in CSF are derived from serum with the exceptions of transthyretin (prealbumin) and transferrin that can come from the choroid plexus as well as β and γ trace proteins, tau protein (tau fraction, modified transferrin), glial fibrillary acidic protein, and myelin basic protein which can be synthesized by the spinal cord (Vernau et al., 2008; Wichmann et al., 2022).

The pathophysiological roles of APP in CNS have been linked to its sequential cleavage by β-secretase and γ-secretase through an amyloidogenic processing pathway to generate Aβ (Zhang et al., 2011), a proximal cause of AD. An increase in the ratio of the longer ($A\beta_{4X\text{-}42}$) neuronally-derived form compared to the more abundant shorter ($A\beta_{4X\text{-}40}$) forms is often used as a biomarker for AD (Borchelt et al., 1996). A consequence of the actions of β-secretase and γ-secretase as well as other secretases is the production of APP intracellular domain protein (AICD) and soluble forms of APP (sAPP) (Sastre et al., 2001). As these cleavage products can be formed through the amyloidogenic and non-amyloidogenic processing pathways, their presence is not directly correlative to Aβ production. Once cleaved from APP, the AICD can form a complex with Aβ-amyloid precursor protein binding family B member 1 (APBB1) and

KAT5 (Probst et al., 2020; Probst et al., 2021; von Rotz et al., 2004) to modulate gene expression upon translocation into the nucleus (Delport and Hewer, 2022). Of relevance to MRDS, we have reported that levels of APBB1 expression are significantly increased in the cortex of the Chrm1$^{-/-}$ mouse (Dean and Scarr, 2021) confirming a link between Chrm1$^{-/-}$ and APBB1. Given the absence of any data on APP processing in the CNS of people with schizophrenia, we decided to study APP processing by measuring levels of cortical sAPP and Aβ (both Aβ $_{42}$ and $_{40}$) in schizophrenia, inclusive of MRDS and non-MRDS, and non-psychiatric controls.

2.1 Methods

2.1.1 *Tissue Collection and Processing*

CNS tissue for this study was collected after obtaining approval from the Ethics Committee of the Victorian Institute of Forensic Medicine and obtaining written consent from the nearest next-of-kin. A case history review was completed using the Diagnostic Instrument for Brain Studies (DIBS) (Hill et al., 1996) to enable diagnoses to be made by consensus according to DSM-IV criteria (American Psychiatric Association, 1994; Roberts et al., 1998). Using data from the DIBS, duration of illness (DI) was calculated as the time from first hospitalisation to death. Post-mortem interval (PMI) was calculated as the time from death to autopsy; however, where death was not witnessed, tissue was only collected from subjects who had been seen alive up to 5h prior to being found dead and, for those cases, PMI was calculated as the midpoint between the person being found dead and last seen alive. The final dose of antipsychotic drug was recorded and then converted to a dose in chlorpromazine equivalents (Foster, 1989; Woods, 2003) (final recorded antipsychotic drug dose: FRADD).

A critical inclusion criterium for tissue collection was that a cadaver must have been refrigerated within 5h of being found to minimise the impact of tissue processing variables. In all cases, the left hemisphere was collected at autopsy, rapidly processed and frozen to -80^{o}C following a standardised procedure (Dean et al., 1999) by the same individual in a way designed to minimise autolytic effects (Ferrer et al., 2007). The pH of the brain tissue was measured as described previously (Kingsbury et al., 1995) as that measure has been shown to be the best indicator of tissue preservation (Stan et al., 2006). During processing, the tissue was carefully examined to ensure the absence of any pathological changes that would exclude

the diagnosis of schizophrenia. A more extensive microscopic examination of the right hemisphere was undertaken to further ensure the absence of pathologic changes that would exclude the diagnosis of schizophrenia (Velakoulis et al., 2009).

For this study, tissue was collected from the frontal cortex (BA 6: premotor cortex: the caudal portion of the superior frontal gyrus and the middle frontal gyrus extending from the cingulate sulcus on the medial surface to the lateral sulcus on the lateral surface) (Garey, 1994). In addition to comparing data from people with schizophrenia (n = 28) to controls (n = 14), the cases with schizophrenia were divided into those with MRDS (levels of [^{3}H]pirenzepine binding of ≤ 110 fmol / mg ETE in BA 9; n = 14) and non-MRDS ([^{3}H]pirenzepine binding of > 110 fmol / mg ETE in BA 9, n = 14)) (Scarr et al., 2009)

2.1.2 *[^{3}H]pirenzepine binding*

To show that changes in [^{3}H]pirenzepine binding in BA 6 was predominantly restricted to cases with MRDS we measured levels of the binding of that radioligand as described previously using a single-point saturation method (Scarr et al., 2009) which, because of methodological design, gives a good approximation of the density of radioligand binding sites in tissue sections (Rodbard, 1981). Thus, five frozen tissue sections (20mm) were cut from BA6 of each case and incubated with [^{3}H]pirenzepine (15nM) in the presence (non-specific binding: 2 sections) or absence (total binding: 3 sections) of 1.0mM quinuclidinyl xanthene-9-carboxylate hemioxalate in 10mM sodium-potassium phosphate buffer (10mM KH_2PO_4, 10mM Na_2HPO_4, pH7.4) at room temperature for 30 min. Each section was then washed twice for 2 min in ice-cold 10mM sodium potassium phosphate buffer, dipped in ice-cold water and thoroughly dried prior to being fixed overnight in paraformaldehyde fumes in a desiccator. Subsequently,

groups of sections and a set of [^{3}H]micro-scales were apposed to a BAS-TR2025 imaging plate until an image of appropriate intensity was obtained for scanning in a BAS 5000 high-resolution phosphoimager (3 days). The intensity of the phosphoimages were measured by comparison to the intensity of the segments of radioactivity on the [^{3}H]micro-scales using AIS image analysis software. As per our previous studies in different cortical regions (Crook et al., 2001; Dean et al., 2008; Gibbons et al., 2013; Scarr et al., 2009), levels of [^{3}H]pirenzepine binding was found to be homogeneous across the cortical laminae, hence a transcept was placed over all the cortical laminae to enable an integrated measure of radioligand binding across the laminae of the cortex to be measured (Dean et al., 1999). The specific binding of [^{3}H]pirenzepine to each section was calculated as total minus non-specific binding in d.p.m. mg of estimated wet weight tissue equivalents (ETE) and then converted to fmol mg / ETE.

2.1.3 *Soluble Aβ-Amyloid Precursor Protein (sAPP) Western blotting*

Levels of sAPP were measured as described previously (Dean et al., 2019b). Hence, samples of BA 6 from the same block used for radioligand binding were dissected and kept at -80^{o}C until required for Western blotting at which time the samples were partially defrosted on a bed of dry ice to isolate grey matter from white matter and meninges. Gray matter was collected, weighed and added to 5 x weight / volume (wt / vol) phosphate buffered saline containing EDTA-free protease inhibitor cocktail (1:50, Roche, Dee Why, NSW, Australia) and homogenised with 20 x 1s pulses on a sonicator. The resulting soluble proteins were isolated by centrifugation at 100,000 g at 4^{o}C for 30 min and the protein concentration determined using a BCA assay (Pierce, Mt Waverley, VIC, Australia).

Twenty µg of total protein from the soluble fraction of BA 6 were loaded, in triplicate, onto 2 x 4–20% PAGE (Bis-Tris, Invitrogen) and transferred to nitrocellulose membrane using iBlot (Invitrogen). One nitrocellulose membrane was then incubated with mouse anti-APP antibody (1:100, in house 22C11 monoclonal antibody) which has been shown to recognize an epitope within the N-terminal extracellular region of the protein between residues 66 and 81(Hilbich et al., 1993). For this study, the intensity of the prominent diffuse band at ~98kDa (Supplementary Figure 1) was measured as this has been shown to be sAPP (Höfling et al., 2016; Rose et al., 2012). A second membrane was incubated with a mouse anti-β-actin antibody (1:10,000, AbCam). sAPP and β-actin were visualised with ECL (Amersham) on a LAS-3000 Imaging suite and the intensity of the signal from each protein captured using Multi Gauge (Fuji), the intensities of the signal of the two proteins were measured using Image J (National Institutes of Health) (Schindelin et al., 2012) and then normalised to levels of β-actin in the same tissue.

2.1.4 *$A\beta_{40}$ and $A\beta_{42}$ ELISA*

$A\beta_{40}$ and $A\beta_{42}$ levels were determined using the DELFIA® Double Capture ELISA as previously described (George et al., 2004). Thus, cortical tissue (~0.2 g) was homogenized in Tris buffer with saline and proteinase inhibitors (TBS) in a ratio of 1:10 (wt / vol), sonicated and centrifuged at 100,000 g for 1 hr. The resulting supernatant containing the soluble cortical fraction was collected and the pellet (insoluble fraction) was resuspended in TBS by sonication and solubilised with 5M Guanidine HCl (GHCl). Each soluble fraction of cortex was diluted with blocking buffer (0.25% casein or Superblock in phosphate-buffered saline with 0.025% Tween-20) followed by centrifugation at 16,000 g for 20 min. Then 40 µl of each sample, in triplicate, was mixed with 10 µl 2.5M GHCl (final 0.5M) and loaded into a well of a microtitre

plate that also contained Aβ40 and Aβ42 peptide standards, also in triplicate. The guanidine solubilised pellet fractions were first diluted to 0.5M GHCL before loading to the plate. The measurement of levels of $A\beta_{40}$ or $A\beta_{42}$ were made using either microtitre plates coated with either G210 monoclonal antibody (for $A\beta_{40}$) or G211 monoclonal antibody (for $A\beta_{42}$) which were then blocked with 0.5% (w / v) casein/PBS or Superblock/PBS buffer (pH 7.4). After washing the plates, WO2-Biotin was added to each well and the plates were incubated overnight at 4°C. After this incubation, the plates were washed, europium labelled streptavidin added, and then developed with enhancement solution. Analysis was carried out using the Wallac Victor 1420 Multilabel Plate Reader (PerkinElmer, Melbourne, Australia) with excitation at 340nm and emission at 613nm.

2.1.5 *Statistics*

It is difficult to accurately assess the distribution of data in small sample sizes (D'Agostino et al., 1990) but in this study most data sets appeared to fit a normal distribution and hence parametric statistics were used. Thus, significant variations in data from people with schizophrenia and controls were identified using Student's t test. A One-way ANOVA was used to identify variation in data from MRDS, non-MRDS and controls and a post hoc Dunnett's comparison between all groups was used to identify the source of that variation. For non-numeric data, differences in the frequency of variables within groups were identified using the χ^2 test. Relationships between experimental data and continuous data of potential confounds were identified using linear regression assuming a straight line best fit. As these analyses were completed using small data sets, only strong relationships ($r^2 \geq 0.70$) were taken as indicating potential biologically relevant relationships (Cook and Weisberg, 1999). All

analyses were completed using GraphPad Prism vs 9.00 for Windows (GraphPad Software, LaJolla, California USA).

3.0 Results

There were no significant differences in age, sex ratios, CNS pH, PMI or brain weight (wht) between the controls and the people with schizophrenia, MRDS or non-MRDS (Table 1, Supplementary Table 1). There were no significant differences in DI or FRADD between people with MRDS or non-MRDS. The rate of suicide completion was higher in MRDS (43 %) compared to non-MRDS (21 %).

Levels of cortical [^{3}H]pirenzepine binding did not differ with sex (Supplementary Table 2A). Levels of cortical [^{3}H]pirenzepine binding were lower in suicide completers. This was likely due to an over representation of people with schizophrenia in the suicide completers as radioligand binding did not vary with suicide status in schizophrenia (suicide completion: mean ± SEM: 37 ± 12 fmol / mg ETE; deaths by other causes: 48 ± 8.2 fmol / mg ETE; $p = 0.43$). Whilst there was an over-representation of males in this study, levels of cortical [^{3}H]pirenzepine binding did not differ with sex (Supplementary Table 2A).

Levels of cortical sAPP, $A\beta_{40}$ and $A\beta_{42}$ did not vary with sex or suicide status (Supplementary Table 2A). Levels of [^{3}H]pirenzepine binding, sAPP, $A\beta_{40}$ and $A\beta_{42}$ did not strongly correlate with donor age, CNS pH, PMI, brain Wht, DI or FRADD (Supplementary Table 2B). Finally, levels of cortical [^{3}H]pirenzepine binding did not correlate strongly with levels of sAPP ($r^2 = 0.041$, $p = 0.15$), $A\beta_{40}$ ($r^2 = 0.027$, $p = 0.29$) of $A\beta_{42}$ ($r^2 = 0.024$, $p = 0.32$).

Overall, levels of [^{3}H]pirenzepine binding was lower in BA 6 compared to what would be expected in BA 9 (Scarr et al., 2009). As would be predicted, levels of [^{3}H]pirenzepine binding were lower in people with schizophrenia compared to controls (Cohen's $d = 1.86$) (Figure 1;,

Table 1). In addition, compared to controls levels of [^{3}H]pirenzepine binding was markedly lower in BA 6 from people with MRDS (Cohen's *d* = 4.46) but did not differ significantly in people with non-MRDS (Cohen's *d* = 1.16) (Figure 1, Table 1). Compared to controls, levels of sAPP were significantly lower in the cortex of people with schizophrenia (Cohen's *d* = 1.24), MRDS (Cohen's *d* = 0.95) or non-MRDS (Cohen's *d* = 1.50)(Figure 2, Table 1, Supplementary Table 1). By contrast, levels of $A\beta_{40}$ and $A\beta_{42}$ and the ratio of $A\beta_{40}$ / $A\beta_{42}$ did not differ between controls and people with schizophrenia, MRDS or non-MRDS (Figure 2, Table 1, Supplementary Table 1).

4.0 Discussion

Here we report lower levels of sAPP in the frontal cortex from people with schizophrenia which are not specific to those in the MRDS or non-MRDS sub-groups. Changes in levels of APP are not restricted to the frontal cortex in schizophrenia as there are lower levels of that protein in cerebrospinal fluid (CSF) (Hidese et al., 2020) and platelets (Tereshkina et al., 2020) from people with the disorder. In addition, lower levels of APP in the CNS (including CSF and platelets) may have some diagnostic selectivity for schizophrenia as we found no similar changes in the frontal cortex (BA 10 and BA 6) from people with major depressive disorders and bipolar disorders (Dean et al., 2020). We also show there are no changes in levels of $A\beta_{40}$ and $A\beta_{42}$ or the ratio of $A\beta_{40}$ / $A\beta_{42}$ in the frontal cortex from people with schizophrenia. Thus changes in APP metabolism in schizophrenia are different from Alzheimer's disease where an increase in the ratio of $A\beta_{40}$ / $A\beta_{42}$ are evident as a biomarker for the disorder (Borchelt et al., 1996). Our data on sAPP, $A\beta_{40}$ and $A\beta_{42}$ add to our previous data showing that there are no differences in protein levels of β-secretase 1 (BACE1), an enzyme that catalyzes the first step in the formation of Aβ peptide from APP (McDade et al., 2021), in the frontal cortex (BA 6) from people with schizophrenia (Dean et al., 2008).

Based on our data on the cortical transcriptome in people with MRDS (Scarr et al., 2018), this study was undertaken to test the hypothesis that APP was involved in a pathway affected by the molecular pathology of that endophenotype. The data from this study shows that there is evidence of changes in APP processing in the cortex of people with schizophrenia but this is not limited to people with MRDS. APP is known to have important roles in neurogenesis, neurite outgrowth, axon guidance and synaptogenesis (Chau et al., 2023) which are all implicated in the pathophysiology of schizophrenia (Goo et al., 2023; Habela et al., 2016;

Räsänen et al., 2022; Sheu et al., 2019). Interestingly, it has recently been reported that APP has a role in maintaining the temporal balance between self-renewal and differentiation of human neural stem cells and, extrapolating this finding, means APP is important in human-specific temporal patterns of neurogenesis (Shabani et al., 2023). This is relevant to schizophrenia because animal models show that curtailing early gestational cell proliferation results in a schizophrenia-like CNS pathology (Selemon and Zecevic, 2015). It has also been suggested that pruning of axonal collaterals and their synapses during childhood and into adolescence is an important contributor to the pathophysiology of schizophrenia (Hoffman and Dobscha, 1989) and it is therefore relevant that APP has a role in axonal and synaptic pruning (Kallop et al., 2014). Our data would support warrants further investigation into whether APP processing is an indicator of APP having a role in these two temporal related developmental processes that are suggested to be critical to the pathophysiology of schizophrenia (Selemon and Zecevic, 2015).

Our current data has ramifications relating to our hypothesis that APP is involved in the molecular pathology of MRDS (Scarr et al., 2018). This hypothesis was based on an interactome study that suggested that lower levels of CHRM1 in MRDS involved the interaction of APP with ApoE (upstream) and lysine acetyltransferase 5 (KAT5) (downstream) (Figure 3); an interactome that had strong face validity due to ApoE being known as regulator of CNS APP function (Koistinaho et al., 2002). Our current data argues that if there is an MRDS specific component in the interactome it is not apparent at the level of APP processing.

Further support of the interactome we previously identified is that levels of KAT5 mRNA ~~is~~are lower in the cortex of people with schizophrenia compared to controls (Kumar et al., 2019), however it is yet to be determined whether this results in a change in levels of KAT5 protein

or restricted to only people with MRDS. In addition, the level of amyloid β precursor protein binding family B member 1 (APBB1) mRNA is increased in the cortex of Chrm1$^{-/-}$ mice (Dean and Scarr, 2021) which is of relevance due to APBB1 being part of a complex with the amyloid percussor intracellular domain (AICD) and KAT5 (Cortes et al., 1988; Probst et al., 2020; von Rotz et al., 2004) that facilitates the translocation of AICD into the nucleus where it regulates gene expression (Delport and Hewer, 2022). Our data on APBB1 mRNA in the cortex of Chrm1$^{-/-}$ mice argues that the level of AICD, KAT5, APBB1 complex in the cortex may be controlled by CHRM1 signalling which is selectively impaired in people with MRDS (Salah-Uddin et al., 2009). Furthermore, we have reported preliminary data on increased levels of AKT-interacting protein (AKTIP) in the cortex of people with MRDS but not non-MRDS (Snelleksz and Dean, 2022). AKTIP regulates the phosphorylation of AKT Serine/Threonine Kinase 1 (AKT1) by pyruvate dehydrogenase kinase 1 (PDK1) (Khalil et al., 2021) and the level of phosphorylation of AKT1 regulates its activity. With regards to our current data, activation levels of AKT1 signaling has been shown to control levels of APP trafficking (Shineman et al., 2009). Together these data suggest it will be worthwhile investigating the AICD, KAT5, APBB1 complex in schizophrenia and whether this complex may be selectively affected in MRDS.

Relevant to this study is the finding that modulating signalling through CHRM1 has affects on APP processing (Scarpa et al., 2020). This raises the possibility that the breakdown in homeostasis in the pathway we have identified as being significantly disrupted in MRDS could be driven by low levels of CHRM1 signalling in the MRDS sub-group. Arguing against this mechanism are changes in CHRM1 mediated APP processing that results in altered levels of Aβ (Jones et al., 2008; Scarpa et al., 2020) which is inconsistent with our finding of $A\beta_{40}$ and $A\beta_{42}$ being unchanged in the cortex of people with schizophrenia.

This study has a number of limitations. Despite the cohort sizes being typical for many human postmortem CNS studies this is still relatively small. As with all studies that do not involve drug naïve individuals, drug treatment prior to death could be a confound. However, in this study levels of sAPP are not related to the level of antipsychotic drug dose close to the time of death. Furthermore, we have previously shown treating rats chronically with either chlorpromazine or haloperidol does not alter levels of cortical gene expression of *APP* (Dean and Scarr, 2022) and it has been reported that chronic treatment with haloperidol or risperidone does not alter levels of APP protein in the rat cortex (Palotás et al., 2003). These data argue that antipsychotic drug treatments are not the cause of lower levels of cortical sAPP in schizophrenia. Another confound that was not accounted for was smoking; a behaviour more common in people with schizophrenia compared to people without evidence of a psychiatric disorder (Goff et al., 1992; Hughes et al., 1986). Increased inhalation of cigarette smoke in mice results in increased expression of APP in the amygdala and hippocampus (Gutala et al., 2006) and thus, if these findings in mice did translate into similar changes in human cortex after smoking, it is possible the reported lower levels of sAPP may be an underestimate of what would be present in a completely non-smoking cohort of people with the disorder. Moreover, smoking has also been shown to be associated with lower levels of $A\beta_{40}$ and $A\beta_{42}$ in human frontal cortex (Hellström-Lindahl et al., 2004) and thus it is possible levels of $A\beta_{40}$ and $A\beta_{42}$ would have been higher if our study had been completed using non-smokers. It can also be argued the absence of changes in $A\beta_{40}$ and $A\beta_{42}$ in people with schizophrenia, who would be expected to have a higher incidence of smoking, could indicate smoking is not the cause of the changes in sAPP and absence of changes in $A\beta_{40}$ and $A\beta_{42}$ we are reporting.

5.1 Conclusions

In conclusion, we report lower levels of sAPP in the cortex of people with schizophrenia that is independent of MRDS subgrouping and in contrast to our prior findings on mood disorders (Dean et al., 2019a). We also report the APP cleavage products $A\beta_{40}$ and $A\beta_{42}$ are unaltered in the cortex of people with schizophrenia which adds to our previous findings on BACE1 expression also being unchanged in the cortex of people with schizophrenia (Dean et al., 2008). These data argue for a better understanding of APP functions, its proteolytic cleavage in the CNS of people with schizophrenia and its downstream effects on gene expression to elucidate its role in the pathophysiology of the disorder. In addition, given APP is reported to be lower in CSF (Hidese et al., 2020) and platelets (Tereshkina et al., 2020) from patients with schizophrenia, it would be of interest to explore the potential of peripheral APP levels as a biomarker to aid in the diagnosis of the disorder.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Contributions

JD, ES and BD conceptualised the study. JD and QL generated the data on sAPP and Aβ respectively. BD completed all statistical analyses and produced the first draft of the manuscript. All authors contributed to the final version of the manuscript submitted for publication.

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Figure Legends:

Figure 1: Representative autoradiographs, shown as pseudo-coloured images compared to a radioactive microscale, of cortical [^{3}H]pirenzepine binding to a control (A), a person in the MRDS endophenotype (B) and a person not in that endophenotype (C) (N.B. non-specific binding of the radioligand in the presence of 1.0mM quinuclidinyl xanthene-9-carboxylate hemioxalate did not differ from background). (D) Levels (mean ± SEM) of [^{3}H]pirenzepine binding in the dorsolateral prefrontal cortex (BA6 region) from people with schizophrenia, muscarinic receptor deficit schizophrenia (MRDS), non-MRDS and controls.

Figure 2: Levels (mean ± SEM) of soluble β-amyloid precursor protein (sAPP), Aβ 1-40 and Aβ 1-42 in the dorsolateral prefrontal cortex (BA6 region) from people with schizophrenia, muscarinic receptor deficit schizophrenia (MRDS), non-MRDS and controls. Levels of sAPP are expressed as a ratio of β-actin whereas Aβ levels were controlled against total brain weight.

Figure 3: A schematic showing an interactome suggested to be significantly perturbed by changes in gene expression in the prefrontal cortex from patients with MRDS which would lead to the marked down regulation in levels of muscarinic M1 receptor (CHRM1) in that sub-group of people. The schematic shows direction changes at the levels of encoded protein or cleavage products (green increased, red decreased and orange unchanged) for selenium binding protein 1, (SELENBP1), apolipoprotein A1 (APOA1), apolipoprotein E (ApoE), soluble amyloid precursor protein (sAPP) and two forms of Aβ in the CNS or blood from people with

schizophrenia that were not specific to MRDS. In addition, the schematic also highlights the potential importance of the lysine acetyltransferase 5 (KAT5), amyloid β precursor protein binding family B member 1 (APBB1) and~~,~~ APP intracellular domain protein (AICD) on regulating CHRM1 levels as well as a the role for CHRM1 regulating levels of APBB1 and hence affecting the formation of the complex.

Lower Levels of Soluble Aβ-Amyloid Precursor Protein, but not Aβ-amyloid, in the Frontal Cortex in Schizophrenia.

Brian Dean[a,b,*], James Duce[c], Qiao-Xin Li[a,b], Colin L. Masters[a,b] and Elizabeth Scarr[d].

a. The Florey, Parkville, Victoria, Australia.
b. The University of Melbourne of Melbourne Florey Department of Neuroscience and Mental Health, Parkville, Victoria, Australia.
c. MSD Discovery Centre, 120 Moorgate, London, London, UK.
d. The Department of Psychiatry, the University of Melbourne, Parkville, Victoria, Australia.

[*] Corresponding Author: Professor Brian Dean, The Synaptic Biology and Cognition Laboratory, The Florey, 30 Royal Parade, Parkville, Victoria 3052, Australia.

email: brian.dean@florey.edu.au