

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/123027/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Sabherwal, Sophie, Föcking, Melanie, English, Jane A., Fitzsimons, Stephen, Hryniewiecka, Magdalena, Wynne, Kieran, Scaife, Caitriona, Healy, Colm, Cannon, Mary, Belton, Orina, Zammit, Stanley, Cagney, Gerard and Cotter, David R. 2019. ApoE elevation is associated with the persistence of psychotic experiences from age 12 to age 18: Evidence from the ALSPAC birth cohort. *Schizophrenia Research* 209, pp. 141-147. 10.1016/j.schres.2019.05.002

Publishers page: <http://dx.doi.org/10.1016/j.schres.2019.05.002>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



ApoE elevation is associated with the Persistence of Psychotic Experiences from age 11 to age 18: Evidence from the ALSPAC Birth Cohort

Sophie Sabherwal¹, Melanie Föcking¹, Jane A English¹, Stephen Fitzsimons², Magdalena Hryniewiecka¹, Kieran Wynne², Caitriona Scaife², Colm Healy¹, Mary Cannon¹, Orina Belton², Stanley Zammit^{3,4}, Gerard Cagney², David R. Cotter^{*1}

¹Department of Psychiatry, Royal College of Surgeons in Ireland (RCSI), Beaumont Hospital, Dublin, Ireland; ²School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Dublin, Ireland. ³MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, Cardiff, UK; ⁴Centre for Academic Mental Health, Bristol Medical School, University of Bristol, Bristol, UK

To whom correspondence should be addressed; Royal College of Surgeons in Ireland Education & Research Centre, Beaumont Hospital, Beaumont, Dublin 9, Ireland; tel: 01-809-3862, fax: 01-809-3741, e-mail: drcotter@rcsi.ie

Word count of the abstract: 149

Word count of the manuscript: 3780

Number of figures: 2

Number of tables: 2

Supplemental information: Supplemental methods, 1 table, 1 figure

Abstract

Apolipoproteins, which play important roles in lipid metabolism, innate immunity and synaptic signalling, have been implicated in first episode psychosis and schizophrenia. This is the first study to investigate plasma apolipoprotein expression in children with psychotic experiences that persist into adulthood. Here, using semi-targeted proteomic analysis we compared plasma apolipoprotein expression levels in age 11 subjects who reported psychotic experiences at both age 11 and age 18 (n=38) with age-matched controls who only experienced psychotic experiences (PEs) at age 11 (n=38). Participants were recruited from the UK Avon Longitudinal Study of Parents and Children (ALSPAC) cohort who participated in psychiatric assessment interviews at ages 12 and 18. We identified apoE, a marker of Alzheimer's Disease, to be significantly up regulated ($p < 0.003$) in those with persistent psychotic experiences. We confirmed this finding in these samples using ELISA. Our findings indicate elevated plasma apoE in age 11 children who experience PEs is associated with persistence psychotic experiences.

Key words: Psychotic experience; Outcome; Proteomics; ALSPAC; ApoE; Apolipoprotein

1. Introduction

The early detection and treatment of psychosis patients significantly improves their clinical outcome. Consequently, any clinical or biological marker of future psychotic experiences are potentially of great clinical value. However, the clinical characteristics of those at risk of future psychotic disorder, such as those in the at-risk-mental state, ultra-high risk (UHR) and clinical-high risk (CHR) have been found to be of relatively limited predictive value (Welsh & Tiffin ., 2013). Risk calculators have been developed which include neuropsychological, neuroimaging and biological measures (Cannon et al., 2016, Clark et al., 2016).

In relation to biological markers, the field of blood biomarker discovery for disorders of the brain has been legitimized by replicable blood-based biomarker findings in Alzheimer's disease (Ovod et al., 2017, Nabers et al., 2018, Kaneko et al., 2014). This is of huge significance to psychiatry and neurology due to the relative ease of accessibility to patient blood in comparison to other biological samples. In addition, blood confers a significant advantage over other peripheral samples in that it can be taken at any stage during the course of illness, unlike cerebrospinal fluid (CSF) for instance. These factors are driving the search for predictive (risk-calculating) blood biomarkers for central nervous system disorders such as schizophrenia and psychosis (Perkins et al., 2015, Chan et al., 2015).

Altered levels of plasma apolipoproteins have been observed in schizophrenia and first episode psychosis (FEP) and indeed are some of the most replicable findings in blood biomarkers studies of FEP (Sabherwal et al. 2016). Specifically, reduced plasma apolipoprotein A1 (apoA1), a prognostic marker for cardiovascular disease, was found to be reduced in the blood of drug free schizophrenia and FEP patients by six independent

proteomic studies (Sabherwal et al. 2016). Seven other apolipoproteins have also been found to be altered in drug free schizophrenia and FEP subjects (\downarrow apoA-2, \downarrow apoA-4, \downarrow apoC-1, \downarrow apoC-3, \downarrow \uparrow apoD, \uparrow apoH, \downarrow apoL-1) two of which have been replicated (apoA2 and apoH) (Levin et al., 2010, Jaros et al., 2012, Domenici et al., 2010, Chan et al., 2015, Sabherwal et al., 2016, Ramsey et al., 2013, Li et al., 2012). In keeping with these findings, a recent proteomic study of age 11 plasma from subjects who developed an extended broad psychosis phenotype at age 18 showed dysregulation of four apolipoproteins (\downarrow apoA2, \uparrow apoH, \uparrow clu and \uparrow apoA4) (English et al., 2018a). Finally, apoD was found to be elevated in CHR subjects who transitioned to psychosis versus those who did not (Perkins et al., 2015). These changes suggest plasma apolipoprotein dysregulation is associated with not only current psychosis but also with a greater risk of future psychosis.

Apolipoproteins are the main protein component of lipoproteins (chylomicrons, high-density lipoprotein; HDL, intermediate density lipoprotein; IDL, low density lipoprotein; LDL, very low-density lipoprotein; VLDL and lipoprotein (a); Lp(a)). Different apolipoproteins are often associated with specific lipoproteins and can thus be involved in the transport of lipids for degradation (for example, apoA1 bound to HDL) or for deposition (for example, apoB bound to LDL). Many apolipoproteins are implicated in the pathology of cardiovascular disease, metabolic syndrome (Adiels et al. 2008), diabetes mellitus type II (Adiels et al. 2008) via dyslipidemia and stroke (for review on the assembly, structure, function of lipoproteins please see Hoofnagle and colleagues, 2009 (Hoofnagle & Heinecke 2009)).

In the brain apolipoproteins function as lipid transporters, like in the periphery, but have additional roles in response to injury, myelin homeostasis, protection against oxidative

stress and synaptic sprouting. ApoE is considered the major brain apolipoprotein and is the primary transporter of cholesterol in the CNS. ApoA1 and apoJ levels are the next most abundant in the CNS. It is generally thought that the brain does not produce apoA-I and that protein levels in the brain come from circulation (plasma apoA1/HDL levels reflect brain levels (Elliott et al., 2010)). Conversely, passage of apoE through the blood-brain barrier appears to be negligible (Shayo et al., 1997).

In light of the evidence for dysregulated apolipoprotein expression within the blood in psychosis we have undertaken the first study in childhood bloods of those who have persisting psychotic experiences from early (age 11) to late childhood (age 18). Using a cutting edge proteomic approach and unique prospective cohort, we compared expression levels of 11 apolipoproteins between age 11 subjects who reported psychotic experiences at both age 11 and age 18 (n=38) compared to age-matched controls who experienced PEs at age 11 only (n=38), in the ALSPAC cohort.

2. Materials and Methods

2.1 Participants

Samples were obtained from the prospective general population cohort ALSPAC which contains demographic, environmental and clinical data on individuals involved. Written informed consent was given prior to plasma sampling. Case and control samples were retrieved from the ALSPAC archive at the same time, stored under the same conditions, and tested in a “blinded” fashion where samples from the test groups were admixed. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Royal College of Surgeons in Ireland Research Ethics Committee (REC1240). Please see searchable data dictionary on the ALSPAC website for full details on all data obtained (www.bristol.ac.uk/alspac/researchers/access).

2.2 Measures of PE and PD

Psychotic Experiences (PEs) were measured by the semi-structured Psychosis-Like Symptoms (PLIKS) interview at ages 11 and 18. Interviews were conducted in assessment clinics by trained psychology graduates and PEs rated according to the definitions and rating rules from the Schedules for Clinical Assessment in Neuropsychiatry, Version 2.0 (Organisation 1994) as not present, suspected or definite. We defined persistent psychotic experiences (PPE) as subjects who had PEs at age 11 and at age 18.

2.3 Study Design

Here, we conducted a nested case-control study comparing the plasma proteomic profiles at age 11 of those with PEs at age 11 and age 18 (n=38) in comparison to those with PE at

age 11 but not age 18 (n=38). See Table 1 for details on grouping. With regards to psychotropic drug use two subjects were recorded as taking psychotropic drugs. No subjects reported psychotropic drug use at age 11. The present study consisted of analyses of two distinct datasets harbouring mass-spectrometry based proteomic profiles.

2.4 Blood collection

For all ALSPAC participants, blood samples from non-fasting individuals were collected at approximately 12 years of age. Blood was collected in 7.5ml Plasma Lithium-Heparin S-Monovette tubes (Sarstedt). Once collected, samples were stored on ice for a maximum of 90 min until processed. After centrifugation, the plasma was stored in aliquots at -80°C. All samples underwent a single freeze thaw cycle in order to aliquot prior to the study. The standard quality of the plasma samples was ensured by assessing the overall MS protein profile in order to facilitate the identification of outlier protein expression profiles (see Supplementary Figure 1).

2.5 High-Abundance Protein Depletion of Plasma Samples

To improve the dynamic range for proteomic analysis, 40µl of plasma from each case in all samples was immunodepleted of the 14 most abundant proteins (Alpha-1-antitrypsin, A1-acid glycoprotein, Serum Albumin, Alpha2-macroglobulin, Apolipoprotein A-I, Apolipoprotein A-II, Complement C3, Fibrinogen alpha/beta/gamma, Haptoglobin, IgG A, IgG G, IgG M, Transthyretin, and Serotransferrin), using the Agilent Hu14 Affinity Removal System (MARS) coupled to a High Performance Liquid Chromatography (HPLC) system (Levin et al., 2010) (see Supplementary Methods).

2.6 Sample Preparation for Mass Spectrometry

Protein digestion and peptide purification was performed as previously described (English et al., 2015), and is further detailed in Supplementary Methods.

2.7 Proteomic analysis

We used the semi-targeted approach of Data Independent Acquisition (DIA) to target all 23 human apolipoproteins with UniProt identifiers. 12 of these showed poor chromatography and were thus excluded (see bioinformatic analysis), leaving 11. DIA overcomes many of the limitations of untargeted proteomics, for example missing values (Sajic et al., 2015, Liu et al., 2015, Aebersold et al., 2016, Teo et al., 2015). 5 μ l of each sample (5 μ g protein) was injected on the Thermo Scientific Q-Exactive, connected to a Dionex Ultimate 3000 (RSLCnano) chromatography system, and data was acquired in DIA mode. The DIA isolation scheme and multiplexing strategy (MSx) was based on that from Egertson et al., in which five separate 4-m/z isolation windows are analysed per spectrum.

In order to create a spectral library for targeted chromatogram extraction, we used an internal standard for quality control (QC), where an equal aliquot from each protein digest in the experiment was pooled into one sample for use as an internal QC. QC samples were injected in data-dependent acquisition (DDA) mode and was injected three times at the beginning of the MS study to condition the column, and subsequently after every ten injections throughout the experiment to monitor the MS performance. To facilitate accurate prediction of peptide *retention* calculation in SkylineTM for DIA data, protein digests were spiked with the PierceTM Peptide Retention Time Calibration Mixture (4 fmol/ μ l), according to the manufacturers' instructions.

2.8 ApoE quantitation by ELISA

For quantitative detection of human apoE in the non-depleted plasma samples of patients with PPE, and controls, we used Apolipoprotein E Human ELISA Kit (ready-to-use sandwich ELISA) with a sensitivity of 1.5 ng/ml and a detection range of 1.64 ng/ml - 400 ng/ml (ThermoFisher Scientific) in accordance with the manufacturer's instructions. For quantitative detection of human ApoE in the non-depleted plasma samples of patients with PPE, and controls, samples were analysed using a Human Apo E (AD2) ELISA Kit (ready to use sandwich ELISA) from ThermoFisher Scientific in accordance with the manufacturer's instructions. Plasma samples were diluted 1 in 1000 before analysis. Standard curve detection ranged from 1.64ng/mL to 400ng/mL.

2.9 Bioinformatic and statistical analysis

Using the open-source software Skyline we performed targeted spectral library-based extraction of peptides from the data acquired by DIA. In addition, all peptide and fragment ions were visually checked and peak integration performed according to the method used by English and colleagues (see supplementary document from (English et al., 2018a)). All apolipoproteins with Uniprot accession numbers were extracted. Filtration of fragment ions and generation of protein-level data was undertaken in mapDIA (Teo et al., 2015). For a full list of the fragments targeted and quantified, please refer to Supplementary Table 1. See supplementary methods for more detail on data handling. Statistical analysis was performed in SPSS by univariate analysis using one-way ANCOVA, with BMI and Gender included as covariates. There was a significant difference in gender distribution between groups, unlike BMI. We adjusted for BMI as the prevalence of obesity in people with mental illness has

been reported to be higher than the general population and recent evidence suggests low childhood BMI between the ages 7 and 13 years is associated with risk of schizophrenia (Sorensen et al., 2016, Chouinard et al., 2016). Furthermore, apoE has been shown to correlate with BMI (Sofat et al., 2016). FDR correction, at 5% cut-off, was performed using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995).

For the validation experiment statistics were performed using GraphPad Prism. Specifically, a Mann Whitney t-test (non-parametric and two-tailed) was performed.

3. Results

3.1 Proteomic analysis of PPEs

Using a semi-targeted proteomic approach, expression levels of 11 apolipoproteins (APOA1, APOA2, APOA4, APOB, APOC1, APOC2, APOC3, APOC4, APOE, APOH, CLU) were compared between participants with PEs at ages 12 and 18 (n=38) and control subjects who had PEs only at age 11 (n=38; Table 1). No PPE cases or controls were excluded from the bioinformatic analysis. Originally, we sought to compare expression levels of all known apolipoproteins (23), however due to sub-threshold detection levels (APOL2-6, APOD, APOA5, APOO), poor chromatography (APOM, LPA, APOF) or filtration due to variability (APOM, LPA) 12 of these were excluded from our analysis. We identified apoE to be significantly differentially expressed between cases and controls (p-value = 0.003 [Benjamini-Hochberg adjusted p-value $p < 0.03$]; Table 2 and Figure 1). The protein was found to be upregulated in those with persistent psychotic experiences.

Using ELISA we confirmed the MS result of apoE upregulation in PPE group (n=38) compared to control (n=37) (p-value 0.034; Figure 2).

4. Discussion

Apolipoproteins have been shown to be dysregulated in blood, brain and CSF in schizophrenia (Sabherwal et al., 2016, Martins-De-Souza et al., 2010, Dean et al., 2003, Thomas et al., 2001, Huang et al., 2008). Our study is the first to assess and to show changes in apolipoprotein expression levels in the plasma of children at age 11 with psychotic experiences that persisted to age 18. We found that altered expression of plasma apoE at age 11 is associated with the persistence of psychotic experiences at age 18. Because subjects who report psychotic experiences (PEs) are at increased risk not solely for schizophrenia but for other major psychiatric disorders such as depression and anxiety disorders (Rutigliano et al., 2016, Kelleher et al., 2012), our findings are of broad relevance to adult psychiatric syndromes.

Previous studies of neuroleptic naïve and chronic schizophrenia have implicated apoE in the disorder. Increased phosphorylation of an apoE peptide was found in the serum of FEP (antipsychotic naïve) patients compared to healthy controls (Jaros et al., 2012). Further, elevated apoE expression, consistent with our own findings, has been reported in studies involving brain and cerebrospinal fluid of non-medicated schizophrenia patients (Dean et al., 2003, Martins-De-Souza et al., 2010). While there is debate whether the level of apoE in blood reflects the cerebral level, CSF apoE has been estimated at 10-20% of the level in plasma despite their regulation by two parallel but metabolically different compartments (Cruchaga et al., 2012, Ulrich et al., 2013, Mahley, 2016). As such, our finding may reflect enhanced CSF apoE in children who will experience persisting psychotic experiences at ages 12 and 18.

Previous findings are not entirely consistent as plasma apoE was found to be reduced in those with schizophrenia (Dean et al., 2008). Possible explanations for these contrasting results include study population differences relating to past and current antipsychotic drug treatment and age, since these have been shown to affect apoE levels (Vik-Mo et al., 2009, Rasmussen, 2016). Furthermore, apoE was not shown to be altered in the blood of schizophrenia patients in a discovery mass-spectrometry study carried out in blood of subjects with schizophrenia (Yang et al., 2006). However, methodological differences may account for this, such as sensitivity of instrumentation and sampling and depletions methods, and contrasting medication status of subjects with psychotic illness (critically our study included only plasma samples from subjects at age 11 and no psychotropic medication had been prescribed for them).

ApoE is functionally diverse and several mechanisms may underlie the elevation in plasma levels observed in the current study. Its primary function is in the transport and metabolism of dietary lipids, mainly triglycerides in plasma, from the intestines to other locations in the body. Indeed a strong association has been shown between apoE and lipid levels within the brain with speculation that apoE possibly in conjunction with reelin maintains and regulates synaptic plasticity (Beasley et al., 2017). Plasma apoE is synthesized in the liver predominantly and to a lesser degree in circulating macrophages (Mooijaart et al., 2006). Hypertriglyceridemic patients have been found to have higher plasma levels of apoE compared with healthy control subjects (Huang et al., 1998b, Sofat et al., 2016, Vincent-Viry et al., 1998). This may be because increased levels of apoE stimulate VLDL triglyceride production in the liver and impair lipoprotein lipase-mediated lipolysis (Huang et al., 1998b, Huang et al., 1998a). Therefore, our finding that plasma apoE is upregulated in those with

PPEs is consistent with the finding of a recent meta-analysis which showed elevated triglycerides in FEP (Pillinger et al., 2017), and with another study showing TGs are elevated at age 11 among apparently well children who report PEs at age 18 (Pillinger et al., 2017, O'Gorman et al., 2017). Plasma apoE is also involved in cholesterol rich HDL endocytosis in the liver. Total cholesterol levels have been shown to be reduced in FEP which is consistent with our finding in that enhanced apoE may result in increased cholesterol rich HDL breakdown (Pillinger et al., 2017).

ApoE is also thought to play a role in the immune response, specifically in inflammation. Plasma levels of apoE are increased up to 4-fold in response to lipopolysaccharide (LPS) induced inflammation in mice despite sharply decreased apoE gene expression in the liver, macrophages, and extrahepatic tissues (suggesting high plasma apoE levels were not due to increased synthesis in liver tissue (Li et al., 2008)). It has therefore been hypothesized that LPS induced inflammation may result in reduced apoE clearance and accumulation in the blood (Fu et al., 2014). In addition, there is now evidence for complement pathway regulation by apoE through interactions between apoE and complement factor H (CFH) of the alternative complement pathway (Haapasalo et al., 2015). The complement pathway has a prominent role in inflammation (Markiewski and Lambris, 2007, Orsini et al., 2014, Kolev et al., 2014) and has been shown to be dysregulated in plasma prior to the onset of psychosis (English et al., 2018b) and in schizophrenia and FEP bloods (Li et al., 2012, Kopczynska et al., 2017)

Other mechanisms by which apoE could be involved in psychotic experiences are via HPA-axis regulation, blood-brain barrier regulation and its effects on cognition. There is evidence

for apoE levels reflecting HPA-axis stimulation including the finding that dexamethasone, a synthetic glucocorticoid, increased the secretion of apoE from macrophages in plasma (Zuckerman et al., 1993). In addition, cortisol and apoE4 have also been found to interact in cognitive decline during aging in that in the presence of the apoE4 allele cortisol levels are associated more strongly with cognitive scores (Lee et al., 2008). ApoE has been associated with transition to dementia from mild cognitive impairment (Hye et al., 2014). This is relevant to the current study considering the role of stress and HPA-axis dysregulation in psychosis risk (Cotter and Pariante, 2002). ApoE is also relevant to psychosis risk through its impact on the integrity of the blood brain barrier (implicated in schizophrenia) (Pollak et al., 2018). ApoE knockout mice exhibit impaired blood-brain barrier integrity (Fullerton et al., 2001) and knockout mice are also vulnerable to psychosis-related behavioural responses following administration of human NMDAR-AB (Hammer et al., 2014). Finally, plasma apoE levels impact on cognition and synaptic function (Lane-Donovan et al., 2016), findings which may be relevant to the cognitive changes found among CHR subjects who transition to psychotic disorder (disorganized communication, poor social functioning and verbal memory deficits in particular) (Addington et al., 2017) and among subjects who report psychotic experiences at age 11 (processing speed and attention in particular) (Niarchou et al., 2013).

ApoE is involved in other psychiatric disorders. The APOE4 allele has a strong association with Alzheimer's Disease (Kim et al., 2009). Protein expression levels have also been researched in Alzheimer's Disease, dementia and in relation to cognition. While the results of these studies in blood are somewhat inconsistent, most evidence now points to low plasma apoE predicting dementia and low plasma apoE as a biomarker for Alzheimer's

disease (Rasmussen, 2016). Increased plasma apoE has also been shown correlate with severity of suicidal behaviour (Asellus et al., 2016). The authors hypothesized this may be related to the regulation of apoE by stress and the role of stress in suicide severity. The findings in Alzheimer's disease and suicidal patients also raise the question of whether apoE elevation in plasma is a biomarker of psychiatric illness more broadly. Given that the current study focusses on subjects who report PEs (not psychotic disorder) and in the knowledge that PEs in childhood are related to a range of non -psychiatric outcomes, including suicide (Heinze et al., 2018, Kelleher et al., 2014a, Kelleher et al., 2014b), our current findings support the view that elevated plasma apoE is associated with vulnerability to psychiatric disorders generally.

Our study has several weaknesses. Firstly, plasma apoE elevation has not previously been reported in medication-free schizophrenia by mass-spectrometry. This however is because most recent studies have not reported apoE quantitation (Levin et al., 2010, Li et al., 2012, Zhou et al., 2014, Ding et al., 2015). Secondly, apoE in humans has several isoforms and therefore protein identification based on molecular weight (as in proteomic methods) can be confounded by the different forms of apoE (Hussain et al., 1989). However, we used ELISA to confirm our findings and therefore we are confident in the results. Thirdly, we undertook depletion of the 14 most abundant plasma proteins before mass spectrometry analysis, including apoA1 and apoA2 and therefore expression levels of these two proteins should be viewed with caution. Fourthly, our study utilised the uniquely characterised ALSPAC cohorts and we could not access a similar age-matched sample in which we could perform a direct replication. Finally, apoE has also been the target of schizophrenia genetic studies and recent meta-analysis showed no significant contribution of APOE4 allele to

schizophrenia risk (Gonzalez-Castro et al., 2015). However, the APOE polymorphism accounts for just 15-25% of the variability of plasma levels (Rasmussen, 2016). There is also evidence that the APOE4 polymorphism specifically modifies the expression of positive symptoms in schizophrenia (Malhotra et al., 1998).

Here we have found plasma apoE to be upregulated in children at age 11 who report persisting psychotic experiences at age 18, compared to children whose PEs do not persist. This is a novel finding which may provide insight into the pathophysiology of PEs and their outcome. It is consistent with recent evidence that lipid metabolism is important in the early stages of psychosis, such as FEP and UHR (McGorry et al., 2014, Amminger et al., 2010, Smesny et al., 2014). Indeed, plasma apoE elevation may reflect disruption in lipid homeostasis but also disturbed inflammation, cognition, blood-brain barrier homeostasis and/or HPA axis regulation. Future investigations are required to confirm and further elucidate on the relationship of apoE to these processes among those with PEs.

References

- <Chan et al. - 2015 - Development of a blood-based molecular biomarker test for identification of schizophrenia before disease onset-annotated.pdf>.
- ADDINGTON, J., LIU, L., PERKINS, D. O., CARRION, R. E., KEEFE, R. S. & WOODS, S. W. 2017. The Role of Cognition and Social Functioning as Predictors in the Transition to Psychosis for Youth With Attenuated Psychotic Symptoms. *Schizophr Bull*, 43, 57-63.
- AEBERSOLD, R., BENSIMON, A., COLLINS, B. C., LUDWIG, C. & SABIDO, E. 2016. Applications and Developments in Targeted Proteomics: From SRM to DIA/SWATH. *Proteomics*, 16, 2065-7.
- AMMINGER, G. P., SCHAFFER, M. R., PAPAGEORGIOU, K., KLIER, C. M., COTTON, S. M., HARRIGAN, S. M., MACKINNON, A., MCGORRY, P. D. & BERGER, G. E. 2010. Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. *Arch Gen Psychiatry*, 67, 146-54.
- ASELLUS, P., NORDSTROM, P., NORDSTROM, A. L. & JOKINEN, J. 2016. Plasma apolipoprotein E and severity of suicidal behaviour. *J Affect Disord*, 190, 137-142.
- BEASLEY, C. L., HONER, W. G., RAMOS-MIGUEL, A., VILA-RODRIGUEZ, F. & BARR, A. M. 2017. Prefrontal fatty acid composition in schizophrenia and bipolar disorder: Association with reelin expression. *Schizophr Res*.
- CANNON, T. D., YU, C., ADDINGTON, J., BEARDEN, C. E., CADENHEAD, K. S., CORNBLATT, B. A., HEINSEN, R., JEFFRIES, C. D., MATHALON, D. H., MCGLASHAN, T. H., PERKINS, D. O., SEIDMAN, L. J., TSUANG, M. T., WALKER, E. F., WOODS, S. W. & KATTAN, M. W. 2016. An Individualized Risk Calculator for Research in Prodromal Psychosis. *Am J Psychiatry*, 173, 980-988.
- CHAN, M. K., KREBS, M. O., COX, D., GUEST, P. C., YOLKEN, R. H., RAHMOUNE, H., ROTHERMUNDT, M., STEINER, J., LEWEKE, F. M., VAN BEVEREN, N. J., NIEBUHR, D. W., WEBER, N. S., COWAN, D. N., SUAREZ-PINILLA, P., CRESPO-FACORRO, B., MAM-LAM-FOOK, C., BOURGIN, J., WENSTRUP, R. J., KALDATE, R. R., COOPER, J. D. & BAHN, S. 2015. Development of a blood-based molecular biomarker test for identification of schizophrenia before disease onset. *Transl Psychiatry*, 5, e601.
- CHOUINARD, V. A., PINGALI, S. M., CHOUINARD, G., HENDERSON, D. C., MALLYA, S. G., CYPRESS, A. M., COHEN, B. M. & ONGUR, D. 2016. Factors associated with overweight and obesity in schizophrenia, schizoaffective and bipolar disorders. *Psychiatry Res*, 237, 304-10.
- CLARK, S. R., BAUNE, B. T., SCHUBERT, K. O., LAVOIE, S., SMESNY, S., RICE, S. M., SCHAFFER, M. R., BENNINGER, F., FEUCHT, M., KLIER, C. M., MCGORRY, P. D. & AMMINGER, G. P. 2016. Prediction of transition from ultra-high risk to first-episode psychosis using a probabilistic model combining history, clinical assessment and fatty-acid biomarkers. *Transl Psychiatry*, 6, e897.
- COTTER, D. & PARIANTE, C. M. 2002. Stress and the progression of the developmental hypothesis of schizophrenia. *Br J Psychiatry*, 181, 363-5.
- CRUCHAGA, C., KAUWE, J. S., NOWOTNY, P., BALES, K., PICKERING, E. H., MAYO, K., BERTELSEN, S., HINRICHS, A., ALZHEIMER'S DISEASE NEUROIMAGING, I., FAGAN, A. M., HOLTZMAN, D. M., MORRIS, J. C. & GOATE, A. M. 2012. Cerebrospinal fluid APOE levels: an endophenotype for genetic studies for Alzheimer's disease. *Hum Mol Genet*, 21, 4558-71.
- DEAN, B., DIGNEY, A., SUNDRAM, S., THOMAS, E. & SCARR, E. 2008. Plasma apolipoprotein E is decreased in schizophrenia spectrum and bipolar disorder. *Psychiatry Res*, 158, 75-8.
- DEAN, B., LAWS, S. M., HONE, E., TADDEI, K., SCARR, E., THOMAS, E. A., HARPER, C., MCCLEAN, C., MASTERS, C., LAUTENSCHLAGER, N., GANDY, S. E. & MARTINS, R. N. 2003. Increased levels of apolipoprotein E in the frontal cortex of subjects with schizophrenia. *Biological Psychiatry*, 54, 616-622.
- DING, Y. H., GUO, J. H., HU, Q. Y., JIANG, W. & WANG, K. Z. 2015. Protein Biomarkers in Serum of Patients with Schizophrenia. *Cell Biochem Biophys*, 72, 799-805.

- DOMENICI, E., WILLE, D. R., TOZZI, F., PROKOPENKO, I., MILLER, S., MCKEOWN, A., BRITAIN, C., RUJESCU, D., GIEGLING, I., TURCK, C. W., HOLSBOER, F., BULLMORE, E. T., MIDDLETON, L., MERLO-PICH, E., ALEXANDER, R. C. & MUGLIA, P. 2010. Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. *PLoS One*, 5, e9166.
- ELLIOTT, D. A., WEICKERT, C. S. & GARNER, B. 2010. Apolipoproteins in the brain: implications for neurological and psychiatric disorders. *Clin Lipidol*, 51, 555-573.
- ENGLISH, J. A., FAN, Y., FOCKING, M., LOPEZ, L. M., HRYNIEWIECKA, M., WYNNE, K., DICKER, P., MATIGIAN, N., CAGNEY, G., MACKAY-SIM, A. & COTTER, D. R. 2015. Reduced protein synthesis in schizophrenia patient-derived olfactory cells. *Transl Psychiatry*, 5, e663.
- ENGLISH, J. A., LOPEZ, L. M., O'GORMAN, A., FOCKING, M., HRYNIEWIECKA, M., SCAIFE, C., SABHERWAL, S., WYNNE, K., DICKER, P., RUTTEN, B. P. F., LEWIS, G., ZAMMIT, S., CANNON, M., CAGNEY, G. & COTTER, D. R. 2018a. Blood-Based Protein Changes in Childhood Are Associated With Increased Risk for Later Psychotic Disorder: Evidence From a Nested Case-Control Study of the ALSPAC Longitudinal Birth Cohort. *Schizophr Bull*, 44, 297-306.
- ENGLISH, J. A., LOPEZ, L. M., O'GORMAN, A., FOCKING, M., HRYNIEWIECKA, M., SCAIFE, C., SABHERWAL, S., WYNNE, K., DICKER, P., RUTTEN, B. P. F., LEWIS, G., ZAMMIT, S., CANNON, M., CAGNEY, G. & COTTER, D. R. 2018b. Blood-Based Protein Changes in Childhood Are Associated With Increased Risk for Later Psychotic Disorder: Evidence From a Nested Case-Control Study of the ALSPAC Longitudinal Birth Cohort. *Schizophrenia Bulletin*, 44, 297-306.
- FU, P., WANG, A. M., HE, L. Y., SONG, J. M., XUE, J. C. & WANG, C. Q. 2014. Elevated serum ApoE levels are associated with bacterial infections in pediatric patients. *J Microbiol Immunol Infect*, 47, 122-9.
- FULLERTON, S. M., SHIRMAN, G. A., STRITTMATTER, W. J. & MATTHEW, W. D. 2001. Impairment of the blood-nerve and blood-brain barriers in apolipoprotein E knockout mice. *Experimental Neurology*, 169, 13-22.
- GONZALEZ-CASTRO, T. B., TOVILLA-ZARATE, C. A., HERNANDEZ-DIAZ, Y., FRESAN, A., JUAREZ-ROJOP, I. E., BLE-CASTILLO, J. L., LOPEZ-NARVAEZ, L., GENIS, A. & HERNANDEZ-ALVARADO, M. M. 2015. No association between ApoE and schizophrenia: Evidence of systematic review and updated meta-analysis. *Schizophr Res*, 169, 355-368.
- HAAPASALO, K., VAN KESSEL, K., NISSILA, E., METSO, J., JOHANSSON, T., MIETTINEN, S., VARJOSALO, M., KIRVESKARI, J., KUUSELA, P., CHRONI, A., JAUHAINEN, M., VAN STRIJP, J. & JOKIRANTA, T. S. 2015. Complement Factor H Binds to Human Serum Apolipoprotein E and Mediates Complement Regulation on High Density Lipoprotein Particles. *J Biol Chem*, 290, 28977-87.
- HAMMER, C., STEPNIAK, B., SCHNEIDER, A., PAPIOL, S., TANTRA, M., BEGEMANN, M., SIREN, A. L., PARDO, L. A., SPERLING, S., MOHD JOFRY, S., GURVICH, A., JENSEN, N., OSTMEIER, K., LUHDER, F., PROBST, C., MARTENS, H., GILLIS, M., SAHER, G., ASSOIGNA, F., SPALLETTA, G., STOCKER, W., SCHULZ, T. F., NAVE, K. A. & EHRENREICH, H. 2014. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. *Mol Psychiatry*, 19, 1143-9.
- HEINZE, K., LIN, A., NELSON, B., RENIERS, R., UPTHEGROVE, R., CLARKE, L., ROCHE, A., LOWRIE, A. & WOOD, S. J. 2018. The impact of psychotic experiences in the early stages of mental health problems in young people. *BMC Psychiatry*, 18, 214.
- HUANG, J. T., WANG, L., PRABAKARAN, S., WENGENROTH, M., LOCKSTONE, H. E., KOETHE, D., GERTH, C. W., GROSS, S., SCHREIBER, D., LILLEY, K., WAYLAND, M., OXLEY, D., LEWEKE, F. M. & BAHN, S. 2008. Independent protein-profiling studies show a decrease in apolipoprotein A1 levels in schizophrenia CSF, brain and peripheral tissues. *Mol Psychiatry*, 13, 1118-28.
- HUANG, Y., LIU, X. Q., RALL, S. C., JR. & MAHLEY, R. W. 1998a. Apolipoprotein E2 reduces the low density lipoprotein level in transgenic mice by impairing lipoprotein lipase-mediated lipolysis of triglyceride-rich lipoproteins. *J Biol Chem*, 273, 17483-90.

- HUANG, Y., LIU, X. Q., RALL, S. C., JR., TAYLOR, J. M., VON ECKARDSTEIN, A., ASSMANN, G. & MAHLEY, R. W. 1998b. Overexpression and accumulation of apolipoprotein E as a cause of hypertriglyceridemia. *J Biol Chem*, 273, 26388-93.
- HUSSAIN, M. M., ZANNI, E. E., KELLY, M. & ZANNIS, V. I. 1989. Synthesis, modification, and flotation properties of rat hepatocyte apolipoproteins. *Biochim Biophys Acta*, 1001, 90-101.
- HYE, A., RIDDOCH-CONTRERAS, J., BAIRD, A. L., ASHTON, N. J., BAZENET, C., LEUNG, R., WESTMAN, E., SIMMONS, A., DOBSON, R., SATTLECKER, M., LUPTON, M., LUNNON, K., KEOHANE, A., WARD, M., PIKE, I., ZUCHT, H. D., PEPIN, D., ZHENG, W., TUNNICLIFFE, A., RICHARDSON, J., GAUTHIER, S., SOININEN, H., KLOSZEWSKA, I., MECOCCHI, P., TSOLAKI, M., VELLAS, B. & LOVESTONE, S. 2014. Plasma proteins predict conversion to dementia from prodromal disease. *Alzheimers Dement*, 10, 799-807 e2.
- JAROS, J. A., MARTINS-DE-SOUZA, D., RAHMOUNE, H., ROTHERMUNDT, M., LEWEKE, F. M., GUEST, P. C. & BAHN, S. 2012. Protein phosphorylation patterns in serum from schizophrenia patients and healthy controls. *J Proteomics*, 76 Spec No., 43-55.
- KANEKO, N., NAKAMURA, A., WASHIMI, Y., KATO, T., SAKURAI, T., ARAHATA, Y., BUNDO, M., TAKEDA, A., NIIDA, S., ITO, K., TOBA, K., TANAKA, K. & YANAGISAWA, K. 2014. Novel plasma biomarker surrogating cerebral amyloid deposition. *Proc Jpn Acad Ser B Phys Biol Sci*, 90, 353-64.
- KELLEHER, I., CEDERLOF, M. & LICHTENSTEIN, P. 2014a. Psychotic experiences as a predictor of the natural course of suicidal ideation: a Swedish cohort study. *World Psychiatry*, 13, 184-8.
- KELLEHER, I., DEVLIN, N., WIGMAN, J. T., KEHOE, A., MURTAGH, A., FITZPATRICK, C. & CANNON, M. 2014b. Psychotic experiences in a mental health clinic sample: implications for suicidality, multimorbidity and functioning. *Psychol Med*, 44, 1615-24.
- KELLEHER, I., KEELEY, H., CORCORAN, P., LYNCH, F., FITZPATRICK, C., DEVLIN, N., MOLLOY, C., RODDY, S., CLARKE, M. C., HARLEY, M., ARSENEAULT, L., WASSERMAN, C., CARLI, V., SARCHIAPONE, M., HOVEN, C., WASSERMAN, D. & CANNON, M. 2012. Clinicopathological significance of psychotic experiences in non-psychotic young people: evidence from four population-based studies. *Br J Psychiatry*, 201, 26-32.
- KIM, J., BASAK, J. M. & HOLTZMAN, D. M. 2009. The role of apolipoprotein E in Alzheimer's disease. *Neuron*, 63, 287-303.
- KOLEV, M., LE FRIEC, G. & KEMPER, C. 2014. Complement--tapping into new sites and effector systems. *Nat Rev Immunol*, 14, 811-20.
- KOPCZYNSKA, M., ZELEK, W., TOUCHARD, S., GAUGHRAN, F., DI FORTI, M., MONDELLI, V., MURRAY, R., O'DONOVAN, M. C. & MORGAN, B. P. 2017. Complement system biomarkers in first episode psychosis. *Schizophr Res*.
- LANE-DONOVAN, C., WONG, W. M., DURAKOGLUGIL, M. S., WASSER, C. R., JIANG, S., XIAN, X. & HERZ, J. 2016. Genetic Restoration of Plasma ApoE Improves Cognition and Partially Restores Synaptic Defects in ApoE-Deficient Mice. *J Neurosci*, 36, 10141-50.
- LEE, B. K., GLASS, T. A., WAND, G. S., MCATEE, M. J., BANDEEN-ROCHE, K., BOLLA, K. I. & SCHWARTZ, B. S. 2008. Apolipoprotein e genotype, cortisol, and cognitive function in community-dwelling older adults. *Am J Psychiatry*, 165, 1456-64.
- LEVIN, Y., WANG, L., SCHWARZ, E., KOETHE, D., LEWEKE, F. M. & BAHN, S. 2010. Global proteomic profiling reveals altered proteomic signature in schizophrenia serum. *Mol Psychiatry*, 15, 1088-100.
- LI, L., THOMPSON, P. A. & KITCHENS, R. L. 2008. Infection induces a positive acute phase apolipoprotein E response from a negative acute phase gene: role of hepatic LDL receptors. *J Lipid Res*, 49, 1782-93.
- LI, Y., ZHOU, K., ZHANG, Z., SUN, L., YANG, J., ZHANG, M., JI, B., TANG, K., WEI, Z., HE, G., GAO, L., YANG, L., WANG, P., YANG, P., FENG, G., HE, L. & WAN, C. 2012. Label-free quantitative proteomic analysis reveals dysfunction of complement pathway in peripheral blood of

- schizophrenia patients: evidence for the immune hypothesis of schizophrenia. *Mol Biosyst*, 8, 2664-71.
- LIU, Y., BUIL, A., COLLINS, B. C., GILLET, L. C., BLUM, L. C., CHENG, L. Y., VITEK, O., MOURITSEN, J., LACHANCE, G., SPECTOR, T. D., DERMITZAKIS, E. T. & AEBERSOLD, R. 2015. Quantitative variability of 342 plasma proteins in a human twin population. *Mol Syst Biol*, 11, 786.
- MAHLEY, R. W. 2016. Central Nervous System Lipoproteins ApoE and Regulation of Cholesterol Metabolism. *Arteriosclerosis Thrombosis and Vascular Biology*, 36, 1305-1315.
- MALHOTRA, A. K., BREIER, A., GOLDMAN, D., PICKEN, L. & PICKAR, D. 1998. The apolipoprotein E epsilon 4 allele is associated with blunting of ketamine-induced psychosis in schizophrenia - A preliminary report. *Neuropsychopharmacology*, 19, 445-448.
- MARKIEWSKI, M. M. & LAMBRIS, J. D. 2007. The role of complement in inflammatory diseases from behind the scenes into the spotlight. *Am J Pathol*, 171, 715-27.
- MARTINS-DE-SOUZA, D., WOBROCK, T., ZERR, I., SCHMITT, A., GAWINECKA, J., SCHNEIDER-AXMANN, T., FALKAI, P. & TURCK, C. W. 2010. Different apolipoprotein E, apolipoprotein A1 and prostaglandin-H2 D-isomerase levels in cerebrospinal fluid of schizophrenia patients and healthy controls. *World Journal of Biological Psychiatry*, 11, 719-728.
- MCGORRY, P., KESHAVAN, M., GOLDSTONE, S., AMMINGER, P., ALLOTT, K., BERK, M., LAVOIE, S., PANTELIS, C., YUNG, A., WOOD, S. & HICKIE, I. 2014. Biomarkers and clinical staging in psychiatry. *World Psychiatry*, 13, 211-23.
- MOOIJAAART, S. P., BERBEE, J. F., VAN HEEMST, D., HAVEKES, L. M., DE CRAEN, A. J., SLAGBOOM, P. E., RENSEN, P. C. & WESTENDORP, R. G. 2006. ApoE plasma levels and risk of cardiovascular mortality in old age. *PLoS Med*, 3, e176.
- NABERS, A., PERNA, L., LANGE, J., MONS, U., SCHATNER, J., GULDENHAUPT, J., SAUM, K. U., JANELIDZE, S., HOLLECZEK, B., RUJESCU, D., HANSSON, O., GERWERT, K. & BRENNER, H. 2018. Amyloid blood biomarker detects Alzheimer's disease. *EMBO Mol Med*, 10.
- NIARCHOU, M., ZAMMIT, S., WALTERS, J., LEWIS, G., OWEN, M. J. & VAN DEN BREE, M. B. 2013. Defective processing speed and nonclinical psychotic experiences in children: longitudinal analyses in a large birth cohort. *Am J Psychiatry*, 170, 550-7.
- O'GORMAN, A., SUVITAIVAL, T., AHONEN, L., CANNON, M., ZAMMIT, S., LEWIS, G., ROCHE, H. M., MATTILA, I., HYOTYLAINEN, T., ORESIC, M., BRENNAN, L. & COTTER, D. R. 2017. Identification of a plasma signature of psychotic disorder in children and adolescents from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort. *Transl Psychiatry*, 7, e1240.
- ORSINI, F., DE BLASIO, D., ZANGARI, R., ZANIER, E. R. & DE SIMONI, M. G. 2014. Versatility of the complement system in neuroinflammation, neurodegeneration and brain homeostasis. *Front Cell Neurosci*, 8, 380.
- OVOD, V., RAMSEY, K. N., MAWUENYEGA, K. G., BOLLINGER, J. G., HICKS, T., SCHNEIDER, T., SULLIVAN, M., PAUMIER, K., HOLTZMAN, D. M., MORRIS, J. C., BENZINGER, T., FAGAN, A. M., PATTERSON, B. W. & BATEMAN, R. J. 2017. Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimers Dement*, 13, 841-849.
- PERKINS, D. O., JEFFRIES, C. D., ADDINGTON, J., BEARDEN, C. E., CADENHEAD, K. S., CANNON, T. D., CORNBLATT, B. A., MATHALON, D. H., MCGLASHAN, T. H., SEIDMAN, L. J., TSUANG, M. T., WALKER, E. F., WOODS, S. W. & HEINSEN, R. 2015. Towards a psychosis risk blood diagnostic for persons experiencing high-risk symptoms: preliminary results from the NAPLS project. *Schizophr Bull*, 41, 419-28.
- PILLINGER, T., BECK, K., STUBBS, B. & HOWES, O. D. 2017. Cholesterol and triglyceride levels in first-episode psychosis: systematic review and meta-analysis. *Br J Psychiatry*, 211, 339-349.
- POLLAK, T. A., DRNDARSKI, S., STONE, J. M., DAVID, A. S., MCGUIRE, P. & ABBOTT, N. J. 2018. The blood-brain barrier in psychosis. *Lancet Psychiatry*, 5, 79-92.

- RAMSEY, J. M., SCHWARZ, E., GUEST, P. C., VAN BEVEREN, N. J., LEWEKE, F. M., ROTHERMUNDT, M., BOGERTS, B., STEINER, J. & BAHN, S. 2013. Distinct molecular phenotypes in male and female schizophrenia patients. *PLoS One*, 8, e78729.
- RASMUSSEN, K. L. 2016. Plasma levels of apolipoprotein E, APOE genotype and risk of dementia and ischemic heart disease: A review. *Atherosclerosis*, 255, 145-155.
- RUTIGLIANO, G., VALMAGGIA, L., LANDI, P., FRASCARELLI, M., CAPPUCCIATI, M., SEAR, V., ROCCHETTI, M., DE MICHELI, A., JONES, C., PALOMBINI, E., MCGUIRE, P. & FUSAR-POLI, P. 2016. Persistence or recurrence of non-psychotic comorbid mental disorders associated with 6-year poor functional outcomes in patients at ultra high risk for psychosis. *J Affect Disord*, 203, 101-10.
- SABHERWAL, S., ENGLISH, J. A., FOCKING, M., CAGNEY, G. & COTTER, D. R. 2016. Blood biomarker discovery in drug-free schizophrenia: the contribution of proteomics and multiplex immunoassays. *Expert Rev Proteomics*, 13, 1141-1155.
- SAJIC, T., LIU, Y. & AEBERSOLD, R. 2015. Using data-independent, high-resolution mass spectrometry in protein biomarker research: perspectives and clinical applications. *Proteomics Clin Appl*, 9, 307-21.
- SHAYO, M., MCLAY, R. N., KASTIN, A. J. & BANKS, W. A. 1997. The putative blood-brain barrier transporter for the beta-amyloid binding protein apolipoprotein J is saturated at physiological concentrations. *Life Sciences*, 60, PI115-PI118.
- SMESNY, S., MILLEIT, B., HIPLER, U. C., MILLEIT, C., SCHAFER, M. R., KLIER, C. M., HOLUB, M., HOLZER, I., BERGER, G. E., OTTO, M., NENADIC, I., BERK, M., MCGORRY, P. D., SAUER, H. & AMMINGER, G. P. 2014. Omega-3 fatty acid supplementation changes intracellular phospholipase A2 activity and membrane fatty acid profiles in individuals at ultra-high risk for psychosis. *Mol Psychiatry*, 19, 317-24.
- SOFAT, R., COOPER, J. A., KUMARI, M., CASAS, J. P., MITCHELL, J. P., ACHARYA, J., THOM, S., HUGHES, A. D., HUMPHRIES, S. E. & HINGORANI, A. D. 2016. Circulating Apolipoprotein E Concentration and Cardiovascular Disease Risk: Meta-analysis of Results from Three Studies. *PLoS Med*, 13, e1002146.
- SORENSEN, H. J., GAMBORG, M., SORENSEN, T. I. A., BAKER, J. L. & MORTENSEN, E. L. 2016. Childhood body mass index and risk of schizophrenia in relation to childhood age, sex and age of first contact with schizophrenia. *Eur Psychiatry*, 34, 64-69.
- TEO, G., KIM, S., TSOU, C. C., COLLINS, B., GINGRAS, A. C., NESVIZHISKII, A. I. & CHOI, H. 2015. mapDIA: Preprocessing and statistical analysis of quantitative proteomics data from data independent acquisition mass spectrometry. *J Proteomics*, 129, 108-120.
- THOMAS, E. A., DEAN, B., PAVEY, G. & SUTCLIFFE, J. G. 2001. Increased CNS levels of apolipoprotein D in schizophrenic and bipolar subjects: implications for the pathophysiology of psychiatric disorders. *Proc Natl Acad Sci U S A*, 98, 4066-71.
- ULRICH, J. D., BURCHETT, J. M., RESTIVO, J. L., SCHULER, D. R., VERGHESE, P. B., MAHAN, T. E., LANDRETH, G. E., CASTELLANO, J. M., JIANG, H., CIRRITO, J. R. & HOLTZMAN, D. M. 2013. In vivo measurement of apolipoprotein E from the brain interstitial fluid using microdialysis. *Molecular Neurodegeneration*, 8.
- VIK-MO, A. O., FERNO, J., SKREDE, S. & STEEN, V. M. 2009. Psychotropic drugs up-regulate the expression of cholesterol transport proteins including ApoE in cultured human CNS- and liver cells. *BMC Pharmacol*, 9, 10.
- VINCENT-VIRY, M., SCHIELE, F., GUEGUEN, R., BOHNET, K., VISVIKIS, S. & SIEST, G. 1998. Biological variations and genetic reference values for apolipoprotein E serum concentrations: results from the STANISLAS cohort study. *Clin Chem*, 44, 957-65.
- YANG, Y., WAN, C., LI, H., ZHU, H., LA, Y., XI, Z., CHEN, Y., JIANG, L., FENG, G. & HE, L. 2006. Altered levels of acute phase proteins in the plasma of patients with schizophrenia. *Anal Chem*, 78, 3571-6.

- ZHOU, N., WANG, J., YU, Y., SHI, J., LI, X., XU, B. & YU, Q. 2014. Mass spectrum analysis of serum biomarker proteins from patients with schizophrenia. *Biomed Chromatogr*, 28, 654-9.
- ZUCKERMAN, S. H., EVANS, G. F. & O'NEAL, L. 1993. Exogenous glucocorticoids increase macrophage secretion of apo E by cholesterol-independent pathways. *Atherosclerosis*, 103, 43-54.

Figure Legends

Figure 1: Protein expression changes determined by mass-spectrometry for apoE. Increased expression in persistent psychotic experiences group (38) versus the non-persistent group (38) (p-value 0.003[Benjamini-Hochberg adjusted p-value $p < 0.03$])

Figure 2: Plasma samples were analysed using a human ApoE ELISA. The graph is representative of plasma from 38 Control Subjects and 37 Persistent Psychotic Experience (PPE) Subjects. Statistical analysis was performed using a non-parametric Mann Whitney T-test. Statistical significance was considered when $*p < 0.05$ (p-value 0.0364).

Tables legends

Table 1: Descriptive information for ALSPAC subjects.

For Gender F = Female, M = Male. Body Mass Index (BMI) at age 11 is reported, where missing BMI variables were replaced with the mean according to gender. PLIKS at age 11 and age 18 are reported. For Depression a binary outcome was reported: individuals with CIS-R scores ≥ 7 as depression (D) and <7 as no depression (ND).

Table 2. Differential Protein Expression in PPE. Semi-targeted proteomic analysis of 12 biomarker candidates in the PPE cohort of cases (n=38) and controls (n=38). Protein level data was assessed for significance and Apolipoprotein E and Clusterin were identified as significantly differentially expressed between cases and controls following adjustment for gender, BMI and multiple comparisons. The protein ID (accession), protein name, gene name, unadjusted and adjusted p-values, fold change (FC) in disorder, and benjamini-hochberg adjusted p-values with FDR cut-off of 0.05 values are listed for all 12 proteins profiled. Proteins are sorted by p-value.

Figure 1

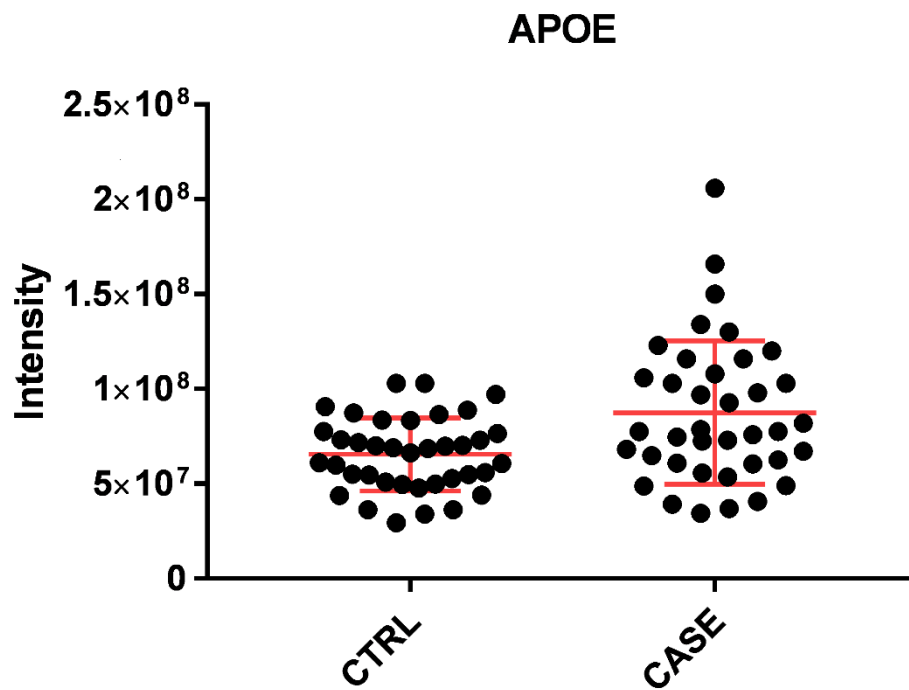


Figure 1: Protein expression changes determined by mass-spectrometry for apoE. Increased expression in persistent psychotic experiences group (38) versus the non-persistent group (38) (p-value 0.003[Benjamini-Hochberg adjusted p-value $p < 0.03$]).

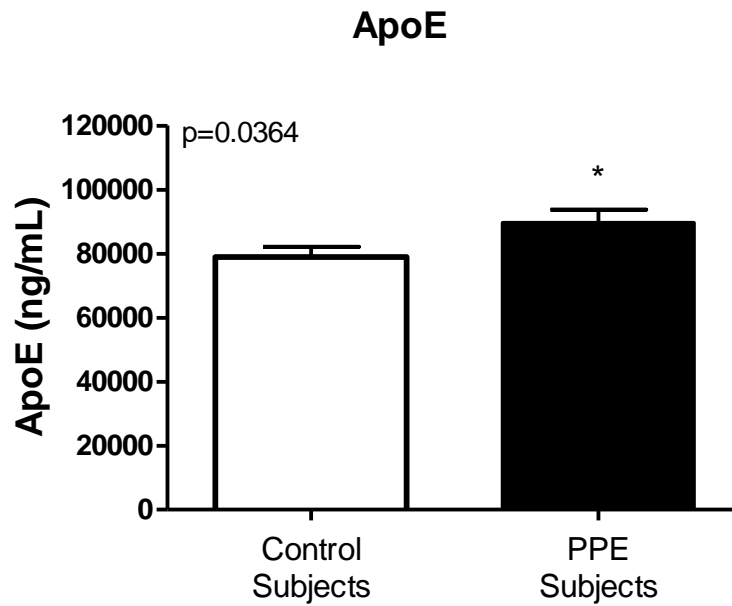


Figure 2: Plasma samples were analysed using a human ApoE ELISA. The graph is representative of plasma from 38 Control Subjects and 37 Persistent Psychotic Experience (PPE) Subjects. Statistical analysis was performed using a non-parametric Mann Whitney T-test. Statistical significance was considered when $*p < 0.05$ (p-value 0.0364).

Table 1: Descriptive information for ALSPAC subjects.

For Gender F = Female, M = Male. Body Mass Index (BMI) at age 11 is reported, where missing BMI variables were replaced with the mean according to gender. PLIKS at age 11 and age 18 are reported. For Depression a binary outcome was reported: individuals with CIS-R scores ≥ 7 as depression (D) and <7 as no depression (ND).

	Persisting Psychotic Experiences () Study	
	Cases (PE12=Def/Sus, PE18=Def)	Controls (PE12=Def/Sus, PE18=0)
Proteomics Study	38	38
Gender	26F, 11M	23F, 15M
BMI at age 11 Mean (StdDev)	18.67 (3.55)	18.46 (2.77)
Ethnicity	32W, 1NW, 4 NA	36W, 2NW
Pliks at age 11	20 Sus, 17 Def	27 Sus, 11 Def
Pliks at age 18	37 Definite	38 None
Social Economic Status	18NM, 12M, 7 NA	26NM, 12M
Depression at age 18	5ND, 30D, 2NA	20ND, 16D, 2NA
Received medication for hallucinations/delusions at age 18	2 Yes, 35 NA	NA
Delusions only happened when within 2 hours of drinking alcohol/taking drugs <i>(Definitely Present/Not Present/NA)</i>	1/18/18	NA

Table 2. Differential Protein Expression in PPE. Semi-targeted proteomic analysis of 12 biomarker candidates in the PPE cohort of cases (n=38) and controls (n=38). Protein level data was assessed for significance and Apolipoprotein E and Clusterin were identified as significantly differentially expressed between cases and controls following adjustment for gender, BMI and multiple comparisons. The protein ID (accession), protein name, gene name, unadjusted and adjusted p-values, fold change (FC) in disorder, and benjamini-hochberg adjusted p-values with FDR cut-off of 0.05 values are listed for all 12 proteins profiled. Proteins are sorted by p-value.

<u>Protein ID</u>	<u>Protein name</u>	<u>Gene name</u>	<u>P-value^a</u>	<u>FC (case/control)</u>	<u>FDR</u>
<u>P02649</u>	Apolipoprotein E	Apo-E	0.0027	1.3361	0.0297
<u>P10909</u>	Clusterin/Apolipoprotein J	Clus	0.0343	1.1446	0.1886
<u>P02749</u>	Apolipoprotein H	Apo-H	0.0533	0.7826	0.1953
<u>P02656</u>	Apolipoprotein C3	Apo-C3	0.1882	0.8850	0.5176
<u>P04114</u>	Apolipoprotein B	Apo-B	0.2026	1.0503	0.4458
<u>P06727</u>	Apolipoprotein A4	Apo-A4	0.4254	1.0726	0.7800
<u>P02655</u>	Apolipoprotein C2	Apo-C2	0.3374	1.1395	0.5302
<u>P02652</u>	Apolipoprotein A2	Apo-A2	0.6209	0.8104	0.8538
<u>P55056</u>	Apolipoprotein C4	Apo-C4	0.7065	0.9130	0.8634
<u>P02647</u>	Apolipoprotein A1	Apo-A1	0.9781	0.9910	1.0759
<u>P02654</u>	Apolipoprotein C1	Apo-C1	0.8787	0.9998	0.8787

^a BMI and gender adjusted p-value

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. This publication is the work of the authors and will serve as guarantors for the contents of this paper. The authors thank Prof Matthias Wilm and the Mass Spectrometry Core Facility at UCD Conway Institute, UCD, for support in the development of our proteomic workflows. In addition, we would like to thank everyone at the MacCoss Lab of Biological Mass Spectrometry, University of Washington, and everyone at the H. Choi Lab, National University of Singapore, for support and access to Skyline and mapDIA, respectively. The authors report no financial interests or potential conflicts of interest.

Conflict of Interest

No conflict of interest to declare.

Contributors

David Cotter designed the study and David Cotter, Gerard Cagney, Jane English and Melanie Föcking wrote the protocol. Sophie Sabherwal generated the hypothesis with regards to protein targets (apolipoproteins). Stephen Fitzsimons and Orina Belton performed the ELISA. Sophie Sabherwal managed the literature searches and analyses. David Cotter and Sophie Sabherwal undertook the statistical analysis, and Sophie Sabherwal wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Funding body agreements and policies

This research was supported by the Irish Health Research Board through a Clinician Scientist Award to DC. ALSPAC is supported The UK Medical Research Council and Wellcome (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. SZ is supported by the NIHR Biomedical Research Centre at University Hospitals Bristol NHS Foundation Trust and the University of Bristol.