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

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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.

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 (↓apoA-2, ↓apoA-4, ↓apoC-1, ↓apoC-3, ↓↑apoD, ↑ apoH, ↓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 (↓apoA2, ↑apoH, ↑clu and ↑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, Apolipoptrotein 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). 5µl of each sample (5µg 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 Skyline[TM] for DIA data, protein digests were spiked with the Pierce[TM] Peptide Retention Time Calibration Mixture (4 fmol/µl), 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.

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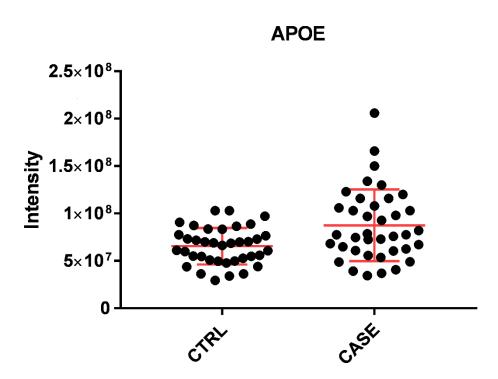
Figure Legends

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 resported: 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 Apolipoportien 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

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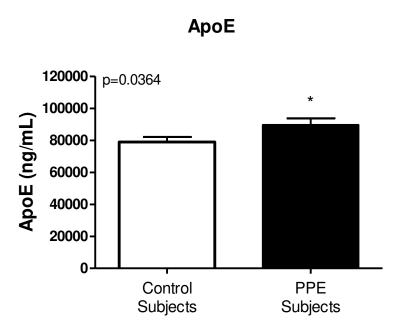(p-value 0.003[Benjamini-Hochberg adjusted p-value p< 0.03]).

Figure 2

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).

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	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 (*Definitely Present/Not Present/NA*)	1/18/18	NA

Table 2

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 Apolipoportien 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.

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

[a] BMI and gender adjusted p-value

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.

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