Psychiatry Research 272 (2019) 380–386

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Relationship between Alzheimer's disease-associated SNPs within the *CLU* gene, local DNA methylation and episodic verbal memory in healthy and schizophrenia subjects

Margarita Alfimova*, Nikolay Kondratyev, Arkadiy Golov, Vera Golimbet

Mental Health Research Center, Moscow 115522, Russia

ARTICLE INFO

Keywords:
Clusterin
ApoJ
Psychosis
Epigenetics
Cognition
Allele-specific methylation
CpG-SNP

ABSTRACT

Genetic variation may impact on local DNA methylation patterns. Therefore, information about allele-specific DNA methylation (ASM) within disease-related loci has been proposed to be useful for the interpretation of GWAS results. To explore mechanisms that may underlie associations between Alzheimer's disease (AD) and schizophrenia risk *CLU* gene and verbal memory, one of the most affected cognitive domains in both conditions, we studied DNA methylation in a region between AD-associated SNPs rs9331888 and rs9331896 in 72 healthy individuals and 73 schizophrenia patients. Using single-molecule real-time bisulfite sequencing we assessed the haplotype-dependent ASM in this region. We then investigated whether its methylation could influence episodic verbal memory measured with the Rey Auditory Verbal Learning Test in these two cohorts. The region showed a complex methylation pattern, which was similar in healthy and schizophrenia individuals and unrelated to haplotypes. The pattern predicted memory scores in controls. The results suggest that epigenetic modifications within the *CLU* locus may play a role in memory variation, independent of ASM.

1. Introduction

Genome-wide association studies (GWAS) have recently identified DNA sequence variants associated with common neuropsychiatric disorders. Functional and clinical consequences of these variants are being investigated with a variety of methods. Yet, little is known about how they confer disease susceptibility. It is proposed that, since genetic variation may have a substantial impact on local DNA methylation patterns, the interpretation of GWAS results can be improved by using information about allele-specific DNA methylation (hereafter ASM) and other allele-specific epigenetic modifications within disease-related loci (Do et al., 2016; Gagliano et al., 2016; Hutchinson et al., 2014; Meaburn et al., 2010). Methylation of CpG dinucleotides in gene promoters usually leads to repression of transcription, while that in exons and introns may regulate splicing (Kader et al., 2018). In this way, ASM, in which the local DNA sequence dictates the methylation status of nearby CpGs, can impact upon disease risk and symptom variability.

Here, we explored DNA methylation within the clusterin gene (*CLU*) for ASM and associations with episodic verbal memory in non-clinical and schizophrenia samples. Clusterin (apoliprotein J) is implicated in many cellular processes, including apoptosis, complement regulation, lipid transport, membrane protection, and cell-cell interactions (Karch and Goate, 2015; Rohne et al., 2016). Several single nucleotide polymorphisms (SNPs) in the *CLU* locus reached genome-wide significance in GWAS of Alzheimer's disease (AD) (Harold et al., 2009; Lambert et al., 2009, 2013; Zhang et al., 2016) and schizophrenia (Pardiñas et al., 2018; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Although data on familial coaggregation of AD and schizophrenia are mixed (Rohde et al., 2016), some other findings suggest that these disorders may have common etiological and pathophysiological factors (for review see (Garcez et al., 2015)). Investigation of the *CLU* polymorphism, one of the shared risk factors, might shed light on mechanisms underlying the two conditions and their cognitive symptoms. Verbal memory is one of the most affected cognitive domains in both AD and schizophrenia (Cirillo and Seidman, 2003; Minati et al., 2009) and represents their cognitive endophenotype (Gur et al., 2007; Pedraza et al., 2014). This makes it an interesting trait to explore the *CLU* role in the development of these neuropsychiatric disorders. The *CLU* role in memory variation may also be important in its own right. Recent findings (De Sanctis et al., 2018) indicate that genes, which influence memory, are numerous and widespread in the brain, with some of them being expressed outside the classical memory networks. Moreover, while deletion/silencing of the majority of these genes induces memory deficit, few genes are coupled

* Corresponding author.
E-mail address: m.alfimova@gmail.com (M. Alfimova).

https://doi.org/10.1016/j.psychres.2018.12.134
Received 21 May 2018; Received in revised form 16 October 2018; Accepted 24 December 2018
Available online 27 December 2018
0165-1781/

with memory enhancement after their deletion. Further research of the gene-memory relationships may thus lead to the discovery of new memory-enhancing drugs.

Symptomatic consequences of schizophrenia risk *CLU* variants are largely unknown. At the same time, AD associated SNPs have been shown to influence cognition, including episodic memory, in AD patients (Barral et al., 2012; Pedraza et al., 2014), elderly and oldest old (Ferencz et al., 2014; Mengel-From et al., 2011, 2013; Pedraza et al., 2014; Sapkota et al., 2016), and young and middle-aged adults (Bressler et al., 2017; Stevens et al., 2014). In addition, the AD risk *CLU* genotype is likely related to preclinical changes in the structure and neurophysiology of the hippocampus, a memory-relevant brain area (Lancaster et al., 2015; Ponomareva et al., 2013; Yang et al., 2016). Finally, the AD risk SNPs seem to associate with some schizophrenia-related impairments. The frequency of the AD risk allele C of rs11136000, the strongest GWAS signal for AD in the *CLU* locus, was increased in schizophrenia patients with negative symptoms (Zhou et al., 2010). An association between rs9331896, another AD GWAS signal in the *CLU* gene, and all dementia except vascular one (Nordestgaard et al., 2018) is also noteworthy since schizophrenia patients are at higher risk of dementia (Ribe et al., 2015). Together, these associations suggest a diagnostically unspecific effect of the *CLU* gene on verbal memory and other mental functions. It can be speculated that its influence occurs early in the lifespan, with different alleles making an individual susceptible or resistant to cognitive impairments in late life or in case of disease. In line with this hypothesis, genetic risk (a polygenic score) for AD was shown to affect verbal memory and the hippocampal volume in childhood (Axelrud et al., 2018).

The present study was based on the hypothesis of ASM influence on disease risk and symptom variability and aimed to explore a potential mediating role of DNA methylation in associations between the *CLU* polymorphism and verbal memory normal variation and impairments. Previously Chibnik et al. (2015) investigated whether DNA methylation would fall along the causal pathway linking the risk GWAS signals to AD. Using brain samples and a quantitative measure of neuritic amyloid plaque as an AD endophenotype, they showed that local AD-associated methylation changes were not driven by rs11136000. We focused our analysis on DNA methylation in a region between two other index SNPs from AD GWASs: rs9331888 and rs9331896 (Fig. 1). The alleles rs9331888 G and rs9331896 T confer risk for AD (Lambert et al., 2013; Zhang et al., 2016). These SNPs are situated upstream of the intronic rs11136000, being in strong linkage disequilibrium (LD) with the latter (The 1000 Genomes Project Consortium, 2015). The region is of interest because of its potential regulatory role in *CLU* expression, as evidenced by the presence of a DNase hypersensitive cluster (Kent et al., 2002). In addition, rs9331888 lying within one of *CLU* alternative promoters has been shown to associate with expression of the alternative splice form NM_203339 and may influence enhancer function by eliminating binding sites for nuclear factor kappa B and early B-cell factor and generating a new binding site for heat shock factor protein-1 (Szymanski et al., 2011). GWAS results are presumably capturing haplotypes (Bell et al., 2018), and the combined effects of several SNPs in a haplotype should explain individual differences in local methylation better than an index SNP (Do et al., 2016). Given this, we first assessed the haplotype-dependent ASM in the region between rs9331888 and rs9331896 in whole blood samples of healthy individuals and schizophrenia patients, based on single molecule real-time bisulfite sequencing (SMRT-BS) (Yang et al., 2015). Second, we investigated whether haplotypes and local methylation patterns would be associated with episodic verbal memory in each of the two cohorts.

2. Methods

2.1. Sample

Participants were selected from a database of the Mental Health Research Center (MHRC) in Moscow described in (Golimbet et al., 2017). Individuals were included if they were Caucasian, aged 18–45 years, completed at least a secondary school (11 years), and had no history of brain injury, psychoactive medication, and somatic or neurologic conditions that may affect cognitive functions. All the participants provided written informed consent, donated blood samples for DNA extraction, and completed a neuropsychological battery that included the Rey Auditory Verbal Learning Test (RAVLT). The study was conducted according to the principles of the Declaration of Helsinki and approved by the local Ethics Committee.

The sample consisted of 145 individuals. These were 72 healthy subjects without a family history of psychoses (*M* 27.94, *SD* 6.83 years, 53% women). It also included 73 patients with schizophrenia spectrum disorders (mean age 26.99, *SD* 6.72 years, 51% women). Healthy individuals were recruited by word of mouth, mainly from the staff of research institutes and hospitals and university students. They did not receive a participation fee. Those who agreed to participate in the study were asked to complete a questionnaire about his/her individual histories of psychiatric and neurological diagnoses, substance use, and a family history of psychoses. The cases were inpatients from the MHRC or Moscow Psychiatric Hospital No 1. According to the ICD-10, 63 patients were diagnosed with schizophrenia (F20), while the others had schizophrenia spectrum disorders: F21 ($n = 3$), F23 ($n = 3$), F25 ($n = 4$). Mean illness duration was *M* 5.40, *SD* 5.92 years.

Epigenome-wide association studies (EWAS) have shown that smoking-induced methylation could occur in many regions of the human genome (Gao et al., 2015). Thus, smoking is a potential confounder in DNA methylation association studies. To consider smoking in the subsequent analysis, we obtained self-reported data on smoking status from 89% of patients and 78% of controls. 33% of patients and 36% of controls were current or former smokers. The groups did not significantly differ in sex, age and smoking. We did not consider educational years above secondary school because the manifestation of schizophrenia often hinders further education.

2.2. Memory assessment

The RAVLT was administered according to a standard procedure (Schmidt, 1996). In our analysis, we used the RAVLT Immediate score, the sum of words recalled across five learning trials, as an episodic verbal memory index. The Immediate score is a reliable measure, which is sensitive to both AD and schizophrenia-related memory impairments as well as to normal memory variation (Badcock et al., 2011; Estévez-González et al., 2003; Schmidt, 1996). Raw scores were converted to T-scores using the mean and *SD* derived from a larger sample of healthy Russian individuals (Lezheiko and Alfimova, 2017).

2.3. DNA methylation profiling and SNP genotyping

Genomic DNAs were isolated from peripheral blood with the DNeasy Blood and Tissue Kit (Qiagen, USA), and these DNA samples (200 ng) were then treated to obtain bisulfate converted DNA with the EpiGentek Methylamp DNA Modification Kit (Epigentek Group Inc., USA) according to the manufacturers' protocols. To amplify 1316 bp PCR product from the rs9331888 - rs9331896 region of bisulfite converted DNA (hg19 chr8:27467639-27468953, + strand), bisulfite PCR was performed according to SMRT-BS (Yang et al., 2015) with some modifications. The sequencing was performed by the University of Washington PacBio Sequencing Services with PacBio RSII (P6/C4 chemistry, CCS reads) and followed by post- sequencing data preparation and quality control. The CpG sites residing in CpG-SNPs were not included in the methylation analysis. For each of the other 26 CpGs situated within the region the degree of methylation (M) was calculated as the fraction of methylated cytosine residues. The additional methodological details are provided in the Supplementary file.

A haplotype analysis was based on three common SNPs rs2070926,

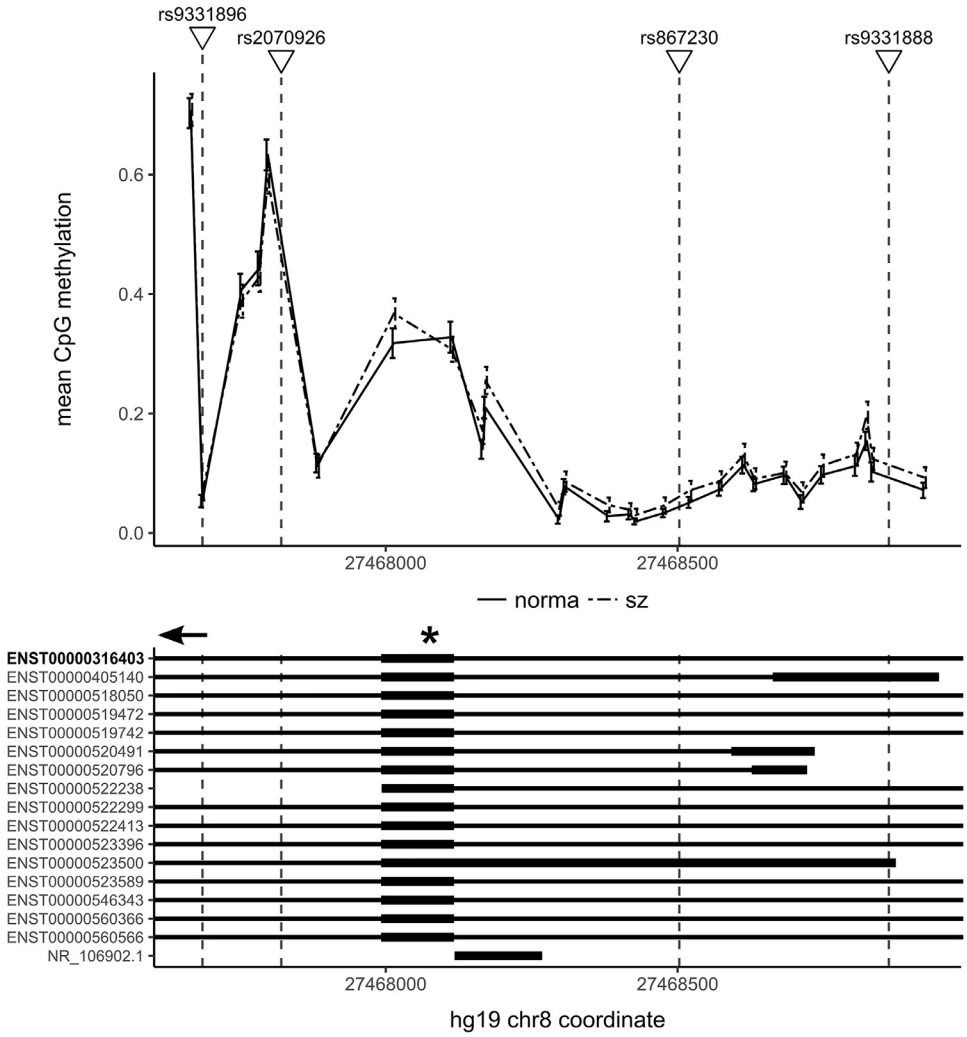

Fig. 1. CpG methylation in the *CLU* alternative promoter region. *Top panel.* Mean methylation levels are shown separately for patients (dashed line) and controls (solid line), with CpG physical positions are presented on the x axis (chromosome 8 position, hg 19). The error bars represent standard errors. The locations of SNPs mentioned in the paper are marked with dashed lines. *Bottom panel.* Different *CLU*transcripts with corresponding Ensembl IDs are presented in relation to the *CLU* locus above, with the name of the main transcript being highlighted in bold. Thick lines represent exons. The asterisk represents the translation start site. The arrow points to the direction of transcription. The location of the mirtron gene *MIR6843* (RefSeq number NR_106902.1) is also shown.

rs867230 and rs9331888 in the target region. Genotypes for each SNP and three major haplotypes consisting of these SNPs (C-C-C, G-A-C, G-A-G) were obtained directly from the sequencing results using a custom perl script. We did not include rs9331896 in either haplotype or methylation analyses. This is a C > T CpG-SNP on + strand, and bisulfite data do not allow to distinguish an unmethylated cytosine from T allele. However, rs9331896 is in almost perfect LD with rs2070926 in European populations, with $D' = 1$ and $R^2 = 0.99$. The allele T of rs9331896 is correlated with the rs2070926 G allele (The 1000 Genomes Project Consortium, 2015). A subject was considered homozygous if more than 90% of his/her reads bore the same allele (haplotype). Since we filtered out heterozygotes with fewer than five reads per haplotype, the sample for the ASM analysis was reduced to 65 patients (of them 16 heterozygotes) and 63 controls (27 heterozygotes). Tests of Hardy–Weinberg equilibrium (HWE) (Rodriguez et al., 2009) showed that all three SNPs conformed to HWE ($p > 0.05$) in both groups. The groups did not differ in genotype distributions (all $p > 0.05$, Table S1).

2.4. Statistical analysis

All analyses were performed with the Statistica and JASP (JASP Team, 2018). Based on a visual analysis we excluded one outlier (a female patient with an extremely hypermethylated pattern). We used beta logit regression to model the relationship between methylation levels of each of the 26 CpGs and haplotypes, considering age, gender, and diagnosis (patient/control).

To this end, numeric values were assigned to the CCC and GAC haplotypes as follows: absent = 0, present = 1. To eliminate 0 and 1 values of methylation without altering the rank order of observations, we compressed the data range as follows: $M' = (M - 0.5)*0.99 + 0.5$ (Triche et al., 2016). The regression analysis was repeated adjusting for smoking. *P*-values were corrected for multiple testing using Benjamini–Hochberg method (with FDR at 5%). To assess haplotype effects on verbal memory, we conducted Bayesian ANCOVAs adjusted for gender and age, separately in controls and patients. To examine if DNA methylation levels would contribute to inter-individual variation of verbal memory, we applied Bayesian correlation analysis and hierarchical linear regression. Prior to the analysis of the relationships between methylation and memory, we excluded five undermethylated CpGs with low variability (M < 0.2 in 90% of the sample) and reduced the methylation data for the other CpGs by means of the principal component (PC) analysis. The PCA is a commonly used technique to reduce dimensionality of correlated data. It helps to remove redundant features, to see patterns in the data and to build simpler models in subsequent analyses**.** Since we intended to use the normal patterns of methylation in this region as a starting point of our analysis and these patterns might be disturbed in schizophrenia, we calculated PCs in the healthy group treating patients as supplementary observations. We applied the Principal Components & Classification Analysis module of Statistica to do that. Next, factor scores were obtained for all subjects, both controls and patients, and we assessed the influence of age, gender, diagnosis, and the “gender X diagnosis” interaction on PCs scores using MANCOVA. Further, we calculated Bayesian Pearson

correlations of PCs with the memory measure, separately in controls and patients. Finally, PCs with at least modest evidence in favor of the correlation were analyzed by means of Bayesian hierarchical linear regression to examine if these correlations are independent of each other and would be retained when controlling for demographic confounders. For each group, a null regression model included age and gender and was compared to the models with these PCs as additional predictors. We used the JASP default priors in all the Bayesian analyses.

3. Results

3.1. ASM

Haplotype frequencies in our samples were as follows: controls, CCC–39 (0.31), GAC–43 (0.34), GAG–44 (0.35); patients, CCC–44 (0.34), GAC–23 (0.18), GAG–63 (0.48). The group difference in the overall haplotype distribution was significant ($\chi 2 = 9.68$, $p = 0.008$). The GAG haplotype was significantly more common (odds ratio [OR], 1.75; 95% confidence interval [CI], 1.06–2.90; $p = 0.028$) and the GAC haplotype was significantly less common (OR, 0.41; 95% CI, 0.23–0.74; $p = 0.003$) in the schizophrenia cases than in the controls.

There were no effects of the haplotypes on DNA methylation. Gender and diagnosis did not impact on the methylation either. A nominally significant effect of age was observed for some of CpGs, but it did not survive the FDR correction. The results did not change when smoking was added into the models. Smoking itself was not a significant predictor of the methylation levels.

3.2. Haplotypes, methylation and verbal memory

Compared to the controls, the schizophrenia subjects had lower memory scores (patients, *M* 35.54, *SD* 12.56; controls, *M* 51.52, *SD* 9.18; $t = 8.18$, $p < 0.001$). There were no haplotype effects on verbal memory.

The methylation pattern was similar in the patient and control groups (Fig. 1). The PC analysis of methylation data revealed four significant PCs (with eigenvalues > 1) that accounted for 61.5% of overall variance in the control group (Table 1). PC1 was related to age (MANCOVA, $F = 5.43$, $p < 0.001$; follow-up ANCOVA, $F = 13.81$, $p < 0.001$). There were no other effects of age, gender, or diagnosis on the PCs.

The Bayes factors (BF_{10} quantifies evidence for the alternative hypothesis relative to the null hypothesis) suggested moderate evidence for positive correlations of verbal memory with PC1 ($r = 0.29$; 95% Credible interval (CI) 0.06–0.48; $BF_{10} = 3.01$) and PC3 ($r = 0.32$; 95% CI 0.09–0.51; $BF_{10} = 5.69$) in the control group (Fig. 2). PC1 reflected methylation levels of most CpGs, with greater loadings for CpGs near rs9331888. Its higher scores meant lower methylation. PC3 had high positive correlations (> 0.5) with methylation levels of two CpGs near the translation start site in exon 2. Higher PC3 scores, therefore, corresponded to higher methylation of these CpGs. The regression analysis showed that the best model included both PCs along with age and gender (Table 2) and explained 21% of the memory variance ($R^2 = 0.21$ vs. $R^2 = 0.11$ for the null model). The estimated BF_{10} suggested that the data were 4.46 times more likely to occur under a model including an effect for methylation than a model without it.

Table 1
Medians and factor loadings based on a principal components analysis for methylation levels of 21 CpGs within the *CLU* gene.

CpG	Median		PC1	PC2	PC3	PC4
	Patients	Controls				
CpG_7666	0.76	0.71				
CpG_7753	0.38	0.40	−0.53	−0.64		
CpG_7783	0.40	0.44	−0.56	−0.64		
CpG_7798	0.60	0.67		−0.73		
CpG_7883	0.05	0.08	−0.65			
CpG_8014	0.38	0.26			0.64	
CpG_8113	0.30	0.30			0.60	
CpG_8166	0.14	0.11	−0.60			
CpG_8171	0.22	0.20	−0.54			
CpG_8307	0.00	0.04	−0.70			
CpG_8521	0.00	0.00	−0.76			
CpG_8573	0.04	0.05	−0.63			
CpG_8613	0.07	0.11	−0.73			
CpG_8632	0.02	0.05	−0.62			0.54
CpG_8684	0.05	0.06	−0.78			
CpG_8713	0.00	0.00	−0.66			0.53
CpG_8748	0.07	0.05	−0.83			
CpG_8806	0.10	0.06	−0.68			
CpG_8824	0.14	0.13	−0.72			
CpG_8834	0.09	0.06	−0.76			
CpG_8923	0.05	0.01	−0.54	0.51		
PC eigenvalue			7.98	2.13	1.48	1.33
% of total variance			37.99	10.16	7.07	6.31

Note: Each CpG name indicates the last four digits of its coordinate on chromosome 8 (27,467,666-27,468,923). Factor loadings < 0.5 are skipped.

4. Discussion

We studied DNA methylation in the region between two AD-associated SNPs within the clusterin gene for ASM and for correlations with episodic verbal memory in middle-aged samples of healthy individuals and schizophrenia patients. The rationale for this study was a hypothesized mediating role of allele-specific DNA methylation, an epigenetic process that can regulate gene expression, in effects of disease-associated genetic variants on phenotypes (Hutchinson et al., 2014; Meaburn et al., 2010). The long-read single-molecule sequencing-based method providing high-resolution quantitative information that is required for a comprehensive and sensitive analysis of ASM was applied. We could observe neither ASM nor haplotypes' effects on verbal memory. In contrast, moderate evidence for correlations between the memory score and DNA methylation pattern within this region was found in healthy individuals.

The *CLU* is a highly expressed gene with complex regulation, for which many mRNA variants and secreted and intracellular forms of the protein are described (Rizzi and Bettuzzi, 2010). The analyzed region contains several sites with different roles in gene expression and translation, including one of alternative promoters, the translation start site, transcription factor binding sites, and exon-intron junctions (Fig. 1). In accord with diverse functions of the studied region, we found its complex methylation pattern, with hypo-, intermediate- and hypermethylated CpGs. The pattern was the same in the patient and control groups suggesting stochastic effects could not explain it. ASM did not influence it either. Evidence indicates that genetic variation at CpG sites is a dominating factor for ASM (Shoemaker et al., 2010). The studied region includes three CpG-SNPs (rs9331896, rs2070926, and rs9331888). We therefore expected to find ASM here, but the haplotype did not affect methylation of the local CpGs, except the CpG-SNPs themselves.

We found that the methylation levels were related to verbal memory in healthy but not schizophrenia individuals. Two sets of CpGs (PCs) might be important. The first one includes most of the low methylated CpG sites, those situated near rs9331888 having the highest loadings. Higher methylation levels of these CpGs predicted a lower memory score. The hypomethylation of CpGs near rs9331888 and its correlation with verbal memory are in accord with the promoter and regulatory roles of this part of the *CLU* gene (Szimansky et al., 2011). The second CpG set (PC3), which correlated with the memory score, included two intermediately methylated CpGs (about 30% in both groups) around the translation start site. Higher methylation levels of these CpGs predicted a higher verbal memory score. The transcribed regions of genes are often heavily methylated, and their methylation level is positively

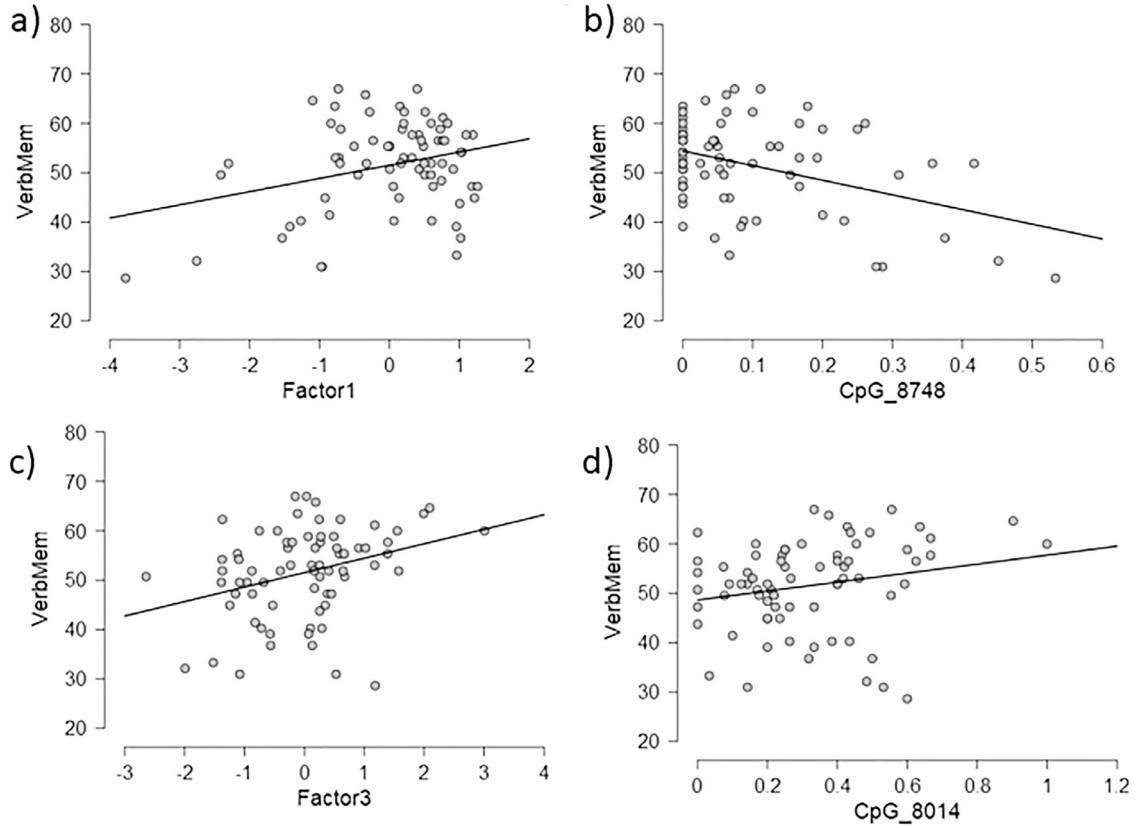

Fig. 2. Relationships between verbal memory (the *y* axis, T-scores) and (a) the first principal component (Factor 1) for methylation data, (b) CpG_8748 that has the largest loading on Factor 1, (c) the third principal component (Factor 3), (d) CpG_8014 that has the largest loading on Factor 3 in the control group.

Table 2
Bayesian regression model for the verbal memory score in healthy individuals.

Parameter	Coefficients			95% credible interval	
	Mean	SD	P(incl/data)	Lower	Upper
Intercept	51.52	0.99	1.00	49.55	53.49
PC1	1.75	1.00	0.60	−0.25	3.74
PC3	2.12	0.94	0.78	0.24	4.01
Gender	−1.52	1.81	1.00	−5.14	2.10
Age	−0.18	0.15	1.00	−0.48	0.13

Note: PC–a principal component. P(incl/data)–posterior inclusion probability.

correlated with the level of gene expression (Kader et al., 2018; Mendizabal et al., 2017) and exon inclusion, intermediate methylation in exons being associated with an intermediate level of exon inclusion (Elliot et al., 2015). The underlying mechanisms remain unknown. Gene body DNA methylation may be a mechanistic consequence of chromatin accessibility levels to methylation enzymes (Mendizabal et al., 2017). On the other hand, there is evidence that gene body DNA methylation increases gene expression (Yang et al., 2014). Regardless of the specific mechanisms, the correlations of verbal memory with methylation of the regulatory/alternative promoter region and the translation start site region may suggest that high and flexible *CLU* expression is needed to protect memory from different detrimental factors arising during the lifespan. This hypothesis is based on the chaperone/scavenging role of secreted clusterin in different tissues (Rohne et al., 2016). Remarkably, GWAS for verbal memory in population-based aging cohorts did not reveal significant signals in the *CLU* locus (Arpawong et al., 2017; Debette et al., 2015). This may be due to the fact that secreted clusterin is primarily important under pathological conditions where it protects the organism and helps to reestablish proteostasis (Rohne et al., 2016). Upregulated *CLU* expression in the brain of AD (Sun et al., 2017) and schizophrenia patients (Pietersen et al., 2014) is consistent with this view. However, we did not observe the relationship between DNA methylation of the *CLU* gene and verbal memory in schizophrenia patients. It can be hypothesized that although verbal episodic memory impairments in schizophrenia share common mechanisms with similar impairments in healthy aging (Silver and Bilker, 2015), there are other disease-specific pathogenic factors that influence memory performance in schizophrenia patients and make the *CLU* effect difficult to detect.

Our results must be viewed in the light of the following limitations. First, we investigated DNA methylation in easily accessible peripheral blood samples. The use of blood as a surrogate for brain in studies of psychiatric disorders is common. This is based on the hypothesis that despite tissue specificity of DNA methylation, there is a certain level of similarity in methylation between blood and brain owing to shared environmental and genetic factors influencing epigenetic processes across various tissues. Research of tissue specificity of methylation at individual CpG sites is underway. Preliminary results suggest that only a minority of variably methylated CpGs correlate between blood and brain (Edgar et al., 2017; Hannon et al., 2015; Walton et al., 2016), and even in the brain itself, DNA methylation is regionally specific (Hannon et al., 2015). Thus, one should be careful when interpreting blood-based methylation data as indicators of brain biology. Considering our study, it is noteworthy that CpG-SNPs are mostly concordant across tissues (Edgar et al., 2017), and the strongest positive correlations found for other CpGs are likely driven by DNA sequence variation (Hannon et al., 2015). Second, whole blood is a heterogeneous collection of different cell types. Its cell composition, which depends on age, is a large source of variability in methylation data (Jaffe and Irizarry, 2014). We restricted our sample to adult individuals not older than 45 years and controlled for age statistically, but our study design did not allow to directly estimate and consider cell-type

composition. Third, antipsychotic drugs may alter DNA methylation at whole-genome and single gene levels (Ovenden et al., 2018). We did not control for this potential confounder because all our patients received complex antipsychotic therapy, which varied during the illness course and even during each episode. However, the medication influence on methylation in the target region is unlikely, as the methylation pattern here is the same in patients and controls. Fourth, our sample may have been underpowered to detect non-obligatory, facilitating (Bell et al., 2018), haplotype effects on methylation. We did not conduct a prospective power analysis because of lack of prior knowledge about methylation levels of different CpGs in the target region and expected effect sizes. Finally, distinguishing cause from effect in epigenetic epidemiology is difficult. Based on the chaperone/scavenging role of clusterin we have hypothesized the causal relationship between methylation within the *CLU* locus and memory. Alternative explanations of the observed correlations are also possible. Specifically, DNA methylation is thought to be affected by a wide range of environmental factors as well as *cis* and *trans* regulatory elements. Some of these factors may independently influence methylation within a certain gene and behavioral phenotypes. Future investigations should consider the potential effects of these factors. Also, research of the transcriptional consequences of the observed methylation patterns in the *CLU* gene could shed light on the mechanisms behind the *CLU*-memory associations.

In summary, we investigated DNA methylation in a region spanning 1.3 kb between two AD-associated SNPs within the *CLU* gene. Previously GWASs identified this gene as a risk locus not only for AD but also for schizophrenia. The region studied showed a complex methylation pattern, which was similar in healthy and schizophrenia individuals. The methylation pattern had a moderate effect on verbal memory in healthy subjects. At the same time, we found no haplotype-specific methylation in this region. Thus, our results do not support the hypothesized mediating role of local DNA methylation in the relationship between genetic variants in the *CLU* gene and episodic memory, one of the AD and schizophrenia endophenotypes. However, they suggest that epigenetic modifications within the *CLU* locus may play a role in memory variability independently of genetic variations.

Funding

This work was supported by the Russian Science Foundation, grant 16-15-00056. The RSF was not involved in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Conflicts of interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.12.134.

References

Arpawong, T., Pendleton, N., Mekli, K., McArdle, J., Gatz, M., Armoskus, C., et al., 2017. Genetic variants specific to aging-related verbal memory: insights from GWASs in a population-based cohort. PLoS ONE 12, e0182448. https://doi.org/10.1371/journal.pone.0182448.

Axelrud, L., Santoro, M., Pine, D., Talarico, F., Gadelha, A., Manfro, G., et al., 2018. Polygenic risk score for Alzheimer's disease: implications for memory performance and hippocampal volumes in early life. Am. J. Psychiatry 175, 555–563. https://doi.org/10.1176/appi.ajp.2017.17050529.

Badcock, J., Dragovic, M., Dawson, L., Jones, R., 2011. Normative data for Rey's auditory verbal learning test in individuals with schizophrenia. Arch. Clin. Neuropsychol. 26, 205–213. https://doi.org/10.1093/arclin/acr005.

Barral, S., Bird, T., Goate, A., Farlow, M., Diaz-Arrastia, R., Bennett, D., et al., 2012. Genotype patterns at PICALM, CR1, BIN1, CLU, and APOE genes are associated with episodic memory. Neurology 78, 1464–1471. https://doi.org/10.1212/wnl.0b013e3182553c48.

Bell, C.G., Gao, F., Yuan, W., Roos, L., Acton, R.J., Xia, Y., et al., 2018. Obligatory and facilitative allelic variation in the DNA methylome within common disease-associated loci. 2018. 9, 8. Nature comm. 9, 8. https://doi.org/10.1038/s41467-017-01586-1.

Bressler, J., Mosley, T., Penman, A., Gottesman, R., Windham, B., Knopman, D., et al., 2017. Genetic variants associated with risk of Alzheimer's disease contribute to cognitive change in midlife: the atherosclerosis risk in communities study. Am. J. Med. Genet. B 174, 269–282. https://doi.org/10.1002/ajmg.b.32509.

Chibnik, L., Yu, L., Eaton, M., Srivastava, G., Schneider, J., Kellis, M., et al., 2015. Alzheimer's loci: epigenetic associations and interaction with genetic factors. Ann. Clin. Trans. Neurol. 2, 636–647. https://doi.org/10.1002/acn3.201.

Cirillo, M., Seidman, L., 2003. Verbal declarative memory dysfunction in schizophrenia: from clinical assessment to genetics and brain mechanisms. Neuropsychol. Rev. 13, 43–77. https://doi.org/10.1023/A:1023870821631.

De Sanctis, C., Bellenchi, G., Viggiano, D., 2018. A meta-analytic approach to genes that are associated with impaired and elevated spatial memory performance. Psychiatry Res. 261, 508–516. https://doi.org/10.1016/j.psychres.2018.01.036.

Debette, S., Ibrahim Verbaas, C., Bressler, J., Schuur, M., Smith, A., Bis, J., et al., 2015. Genome-wide studies of verbal declarative memory in nondemented older people: the cohorts for heart and aging research in genomic epidemiology consortium. Biol. Psychiatry 77, 749–763. https://doi.org/10.1016/j.biopsych.2014.08.027.

Do, C., Lang, C., Lin, J., Darbary, H., Krupska, I., Gaba, A., et al., 2016. Mechanisms and disease associations of haplotype-dependent allele-specific DNA methylation. Am. J. Hum. Genet. 98, 934–955. https://doi.org/10.1016/j.ajhg.2016.03.027.

Edgar, R., Jones, M., Meaney, M., Turecki, G., Kobor, M., 2017. BECon: a tool for interpreting DNA methylation findings from blood in the context of brain. Transl. Psychiatry 7, e1187. https://doi.org/10.1038/tp.2017.171.

Elliott, G., Hong, C., Xing, X., Zhou, X., Li, D., Coarfa, C., et al., 2015. Intermediate DNA methylation is a conserved signature of genome regulation. Nat. Commun. 6, 636. https://doi.org/10.1038/ncomms7363.

Estévez-González, A., Kulisevsky, J., Boltes, A., Otermín, P., García-Sánchez, C., 2003. Rey verbal learning test is a useful tool for differential diagnosis in the preclinical phase of Alzheimer's disease: comparison with mild cognitive impairment and normal aging. Int. J. Geriatr. Psychiatry 18, 1021–1028. https://doi.org/10.1002/gps.1010.

Ferencz, B., Laukka, E., Welmer, A., Kalpouzos, G., Angleman, S., Keller, L., et al., 2014. The benefits of staying active in old age: Physical activity counteracts the negative influence of PICALM, BIN1, and CLU risk alleles on episodic memory functioning. Psychol. Aging 29, 440–449. https://doi.org/10.1037/a0035465.

Gagliano, S., Ptak, C., Mak, D., Shamsi, M., Oh, G., Knight, J., et al., 2016. Allele-skewed DNA modification in the brain: relevance to a schizophrenia GWAS. Am. J. Hum. Genet. 98, 956–962. https://doi.org/10.1016/j.ajhg.2016.03.006.

Gao, X., Jia, M., Zhang, Y., Breitling, L., Brenner, H., 2015. DNA methylation changes of whole blood cells in response to active smoking exposure in adults: a systematic review of DNA methylation studies. Clin. Epigenet. 7, 113. https://doi.org/10.1186/s13148-015-0148-3.

Garcez, M., Falchetti, A., Mina, F., Budni, J., 2015. Alzheimer's disease associated with psychiatric comorbidities. An. Acad. Bras. Cienc. 87 (2 Suppl.), 1461–1473. https://doi.org/10.1590/0001-3765201520140716.

Golimbet, V., Korovaitseva, G., Lezheiko, T., Abramova, L., Kaleda, V., 2017. The serotonin transporter gene 5-HTTLPR polymorphism is associated with affective psychoses but not with schizophrenia: a large-scale study in the Russian population. J. Affect. Disord. 208, 604–609. https://doi.org/10.1016/j.jad.2016.10.020.

Gur, R., Calkins, M., Gur, R., Horan, W., Nuechterlein, K., Seidman, L., Stone, W., 2007. The consortium on the genetics of schizophrenia: neurocognitive endophenotypes. Schizophr. Bull. 33, 49–68. https://doi.org/10.1093/schbul/sbl055.

Hannon, E., Lunnon, K., Schalkwyk, L., Mill, J., 2015. Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. Epigenetics 10, 1024–1032. https://doi.org/10.1080/15592294.2015.1100786.

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M., et al., 2009. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat. Genet. 41, 1088–1093. https://doi.org/10.1038/ng.440.

Hutchinson, J., Raj, T., Fagerness, J., Stahl, E., Viloria, F., Gimelbrant, A., et al., 2014. Allele-specific methylation occurs at genetic variants associated with complex disease. PLOS One 9, e98464. https://doi.org/10.1371/journal.pone.0098464.

Jaffe, A., Irizarry, R., 2014. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. Genome Biol. 15, R31. https://doi.org/10.1186/gb-2014-15-2-r31.

JASP Team, 2018. JASP (Version 0.8.6) [Computer software]. https://jasp-stats.org/.

Kader, F., Ghai, M., Maharaj, L., 2018. The effects of DNA methylation on human psychology. Behav. Brain Res. 346, 47–65. https://doi.org/10.1016/j.bbr.2017.12.004.

Karch, C., Goate, A., 2015. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol. Psychiatry 77, 43–51. https://doi.org/10.1016/j.biopsych.2014.05.006.

Kent, W., Sugnet, C., Furey, T., Roskin, K., Pringle, T., Zahler, A, Haussler, D., 2002. The human genome browser at UCSC. Genome Res. 12 (6), 996–1006. http://genome.ucsc.edu/.

Lambert, J., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M., et al., 2009. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat. Genet. 41, 1094–1099. https://doi.org/10.1038/ng.439.

Lambert, J., Ibrahim-Verbaas, C., Harold, D., Naj, A., Sims, R., Bellenguez, C., et al., 2013. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat. Genet. 45, 1452–1458. https://doi.org/10.1038/ng.2802.

Lancaster, T., Brindley, L., Tansey, K., Sims, R., Mantripragada, K., Owen, M., et al., 2015. Alzheimer's disease risk variant in CLU is associated with neural inefficiency in healthy individuals. Alzheimers Dement. 11, 1144–1152. https://doi.org/10.1016/j.jalz.2014.10.012.

Lezheiko, T., Alfimova, M., 2017. Epigenetic research of cognitive deficit in schizophrenia: some methodological considerations. Zh. Nevrol. Psikhiatr. Im. S. S. Korsakova. 117 (10), 76–80. https://doi.org/10.17116/jnevro201711710176-80.

Meaburn, E., Schalkwyk, L., Mill, J., 2010. Allele-specific methylation in the human genome. Epigenetics 5, 578–582. https://doi.org/10.4161/epi.5.7.12960.

Mendizabal, I., Zeng, J., Keller, T., Yi, S., 2017. Body-hypomethylated human genes harbor extensive intragenic transcriptional activity and are prone to cancer-associated dysregulation. Nucleic Acids Res. gkx020. https://doi.org/10.1093/nar/gkx020.

Mengel-From, J., Christensen, K., McGue, M., Christiansen, L., 2011. Genetic variations in the CLU and PICALM genes are associated with cognitive function in the oldest old. Neurobiol. Aging 32https://doi.org/10.1016/j.neurobiolaging.2010.07.016. e7-554. e11.

Mengel-From, J., Thinggaard, M., Lindahl-Jacobsen, R., McGue, M., Christensen, K., Christiansen, L., 2013. CLU genetic variants and cognitive decline among elderly and oldest old. PLOS One 8, e79105. https://doi.org/10.1371/journal.pone.0079105.

Minati, L., Edginton, T., Grazia Bruzzone, M., Giaccone, G., 2009. Reviews: current concepts in Alzheimer's disease: a multidisciplinary review. Am. J. Alzheimers Dis. Other Demen. 24, 95–121. https://doi.org/10.1177/1533317508328602.

Nordestgaard, L., Tybjærg-Hansen, A., Rasmussen, K., Nordestgaard, B., Frikke-Schmidt, R., 2018. Genetic variation in clusterin and risk of dementia and ischemic vascular disease in the general population: cohort studies and meta-analyses of 362,338 individuals. BMC Med. 16, 39. https://doi.org/10.1186/s12916-018-1029-3.

Ovenden, E., McGregor, N., Emsley, R., Warnich, L., 2018. DNA methylation and antipsychotic treatment mechanisms in schizophrenia: progress and future directions. Prog. Neuropsychopharmacol. Biol. Psychiatry 81, 38–49. https://doi.org/10.1016/j.pnpbp.2017.10.004.

Pardiñas, A., Holmans, P., Pocklington, A., Escott-Price, V., Ripke, S., Carrera, N., et al., 2018. Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. Nat. Genet. 50, 381–389. https://doi.org/10.1038/s41588-018-0059-2.

Pedraza, O., Allen, M., Jennette, K., Carrasquillo, M., Crook, J., Serie, D., et al., 2014. Evaluation of memory endophenotypes for association with CLU, CR1, and PICALM variants in black and white subjects. Alzheimers Dement. 10, 205–213. https://doi.org/10.1016/j.jalz.2013.01.016.

Pietersen, C., Mauney, S., Kim, S., Lim, M., Rooney, R., Goldstein, J., et al., 2014. Molecular profiles of pyramidal neurons in the superior temporal cortex in schizophrenia. J. Neurogenet. 28, 53–69. https://doi.org/10.3109/01677063.2014.882918.

Ponomareva, N., Andreeva, T., Protasova, M., Shagam, L., Malina, D., Goltsov, A., et al., 2013. Age-dependent effect of Alzheimer's risk variant of CLU on EEG alpha rhythm in non-demented adults. Front. Aging Neurosci. 5, 86. https://doi.org/10.3389/fnagi.2013.00086.

Ribe, A., Laursen, T., Charles, M., Katon, W., Fenger-Grøn, M., Davydow, D., et al., 2015. Long-term risk of dementia in persons with schizophrenia: a Danish population-based cohort study. JAMA Psychiatry 72, 1095–1101. https://doi.org/10.1001/jamapsychiatry.2015.1546.

Rizzi, F., Bettuzzi, S., 2010. The clusterin paradigm in prostate and breast carcinogenesis. Endocr. Relat. Cancer 17, R1–R17. https://doi.org/10.1677/erc-09-0140.

Rodriguez, S., Gaunt, T., Day, I., 2009. Hardy–Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am. J. Epidemiol. 169, 505–514. https://doi.org/10.1093/aje/kwn359.

Rohde, C., Agerbo, E., Nielsen, P., 2016. Does schizophrenia in offspring increase the risk of developing alzheimer's dementia. Dement. Geriatr. Cogn. Dis. Extra 6, 361–373. https://doi.org/10.1159/000448395.

Rohne, P., Prochnow, H., Koch-Brandt, C., 2016. The CLU-files: disentanglement of a mystery. Biomol. Concepts 7 (1), 1–15. https://doi.org/10.1515/bmc-2015-0026.

Sapkota, S., Wiebe, S., Small, B., Dixon, R., 2016. Apolipoprotein E and clusterin can magnify effects of personality vulnerability on declarative memory performance in non-demented older adults. Int. J. Geriatr. Psychiatry 31, 502–509. https://doi.org/10.1002/gps.4355.

Schizophrenia Working Group of the Psychiatric Genomics Consortium,Ripke, S., Neale, B.M., Corvin, A., Walters, J.T., Farh, K.-H., et al., 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511, 421–427. https://doi.org/10.1038/nature13595.

Schmidt, M., 1996. Rey Auditory Verbal Learning Test. Western Psychological Services, Los Angeles, CA.

Shoemaker, R., Deng, J., Wang, W., Zhang, K., 2010. Allele-specific methylation is prevalent and is contributed by CpG-SNPs in the human genome. Genome Res. 20, 883–889. https://doi.org/10.1101/gr.104695.109.

Silver, H., Bilker, W., 2015. Similar verbal memory impairments in schizophrenia and healthy aging. Implications for understanding of neural mechanisms. Psychiatry Res. 226, 277–283. https://doi.org/10.1016/j.psychres.2014.12.062.

Stevens, B., DiBattista, A., William Rebeck, G., Green, A., 2014. A gene − brain − cognition pathway for the effect of an Alzheimer's risk gene on working memory in young adults. Neuropsychologia 61, 143–149. https://doi.org/10.1016/j.neuropsychologia.2014.06.021.

Sun, Q., Xie, N., Tang, B., Li, R., Shen, Y., 2017. Alzheimer's disease: from genetic variants to the distinct pathological mechanisms. Front. Mol. Neurosci. 10, 319. https://doi.org/10.3389/fnmol.2017.00319.

Szymanski, M., Wang, R., Bassett, S., Avramopoulos, D., 2011. Alzheimer's risk variants in the clusterin gene are associated with alternative splicing. Transl. Psychiatry 1https://doi.org/10.1038/tp.2011.17. e18-e18.

The 1000 Genomes Project Consortium, 2015. A global reference for human genetic variation. Nature 526 (7571), 68–74. https://doi.org/10.1038/nature15393.

Triche, T., Laird, P., Siegmund, K., 2016. Beta regression improves the detection of differential DNA methylation for epigenetic epidemiology. In: bioRxiv. doi: 10.1101/054643.

Walton, E., Hass, J., Liu, J., Roffman, J., Bernardoni, F., Roessner, V., et al., 2016. Correspondence of DNA methylation between blood and brain tissue and its application to schizophrenia research. Schizophr. Bull. 42, 406–414. https://doi.org/10.1093/schbul/sbv074.

Yang, X., Han, H., De Carvalho, D., Lay, F., Jones, P., Liang, G., 2014. Gene body methylation can alter gene expression and is a therapeutic target in cancer. Cancer Cell 26, 577–590. https://doi.org/10.1016/j.ccr.2014.07.028.

Yang, X., Li, J., Liu, B., Li, Y., Jiang, T., 2016. Impact of PICALM and CLU on hippocampal degeneration. Hum. Brain Mapp. 37, 2419–2430. https://doi.org/10.1002/hbm.23183.

Yang, Y., Sebra, R., Pullman, B., Qiao, W., Peter, I., Desnick, R., et al., 2015. Quantitative and multiplexed DNA methylation analysis using long-read single-molecule real-time bisulfite sequencing (SMRT-BS). BMC Genomics 16, 350. https://doi.org/10.1186/s12864-015-1572-7.

Zhang, S., Li, X., Ma, G., Jiang, Y., Liao, M., Feng, R., et al., 2016. CLU rs9331888 Polymorphism contributes to Alzheimer's disease susceptibility in Caucasian but not east Asian populations. Mol. Neurobiol. 53, 1446–1451. https://doi.org/10.1007/s12035-015-9098-1.

Zhou, Y., Wang, J., Wang, K., Li, S., Song, X., Ye, Y., et al., 2010. Association analysis between the rs11136000 single nucleotide polymorphism in clusterin gene, rs3851179 single nucleotide polymorphism in clathrin assembly lymphoid myeloid protein gene and the patients with schizophrenia in the Chinese population. DNA Cell Biol. 29, 745–751. https://doi.org/10.1089/dna.2010.1075.