Short communication

CSF Apolipoprotein E in attempted suicide

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\textbf{ABSTRACT}

\textbf{Background:} Cholesterol and cholesterol metabolism, involved in continued neural plasticity, has been associated to suicide and suicidal behavior. Apolipoprotein E (ApoE) plays an important role in the cholesterol metabolism. The purpose of this study was to investigate whether ApoE in cerebrospinal fluid was related to severity of suicidal behavior as measured by number of earlier suicide attempts, reversibility/interruptability and violent method of suicide attempt.

\textbf{Methods:} CSF ApoE and 5-hydroxyindolacetic acid (5-HIAA) were measured in 42 medication free suicide attempters. Earlier suicide attempts and the reversibility of suicide attempt method were assessed with the Suicide Intent Scale (SIS) and the Freeman Scale. Suicide attempts were classified according to violence of method.

\textbf{Results:} CSF ApoE levels significantly negatively correlated to the scores on Freeman Reversibility and there was a trend for lower CSF ApoE levels in suicide attempters using a violent method. Patients with at least one earlier suicide attempt (repeaters) showed a trend for higher CSF ApoE levels compared to suicide attempters debuting with suicidal behavior at inclusion in the study. The correlation between CSF ApoE and 5-HIAA was not significant.

\textbf{Limitations:} The main limitations to this study were a relatively small sample size and lack of a healthy control group.

\textbf{Conclusion:} Irreversible suicide attempts, representing a high risk for completed suicide, may be associated with lower level of ApoE in CSF.

1. Introduction

The multifactorial aethiology of suicide and suicidal behaviors includes both proximal and distal life-factors, which are associated with increased risk of suicide through influence on behavioral and emotional traits. The molecular factors correlating to these mediating traits (like impulsive aggression) include cholesterol, which is important for maintaining neural plasticity (Turecki, 2014). Reduced cholesterol content was found in the ventral prefrontal cortex of suicide completers choosing a violent method (Lalovic et al., 2007) and isolated mutations in genes related to cholesterol metabolism have been implicated in violent behavior and suicide (Edgar et al., 2007).

Apolipoprotein E (ApoE) is of potential interest for suicide and suicidal behavior, due to the important role of ApoE in cholesterol metabolism, especially in the central nervous system (CNS). ApoE modulates uptake, release and storage of cholesterol. ApoE in the CNS prevents neuronal death, facilitates the growth of dendrites and formation of new synapses. The presence of ApoE is of special importance after neuronal injury, due to its role in immunomodulation and the reuptake and redistribution of cholesterol (Mahley and Huang, 2012). Furthermore, based on studies in mice, ApoE may play a significant role in regulating hypothalamic-pituitary-adrenal (HPA) axis activity and immune system (Mahley and Huang, 2012), key biological systems implicated in suicidal behavior (Turecki, 2014). Research investigating the association between ApoE and suicidal behavior is scarce.

We have recently reported association between plasma ApoE and severity of suicidal behavior in suicide attempters (Asellus et al., 2016). To our knowledge, there are no studies evaluating the association between ApoE levels in cerebrospinal fluid and suicidal behavior. Based on earlier findings of a relation between plasma ApoE and temporal severity of suicidal behavior, our hypothesis was that repeated suicide attempts may lead to increased levels of ApoE in cerebrospinal fluid due to chronic stress, while a violent, more irreversible/lethal suicide attempt method (indicating a higher level of acute trauma), would be associated to low CSF ApoE. Furthermore, we investigated the association between CSF ApoE and 5-Hydroxyindoleacetic acid (5-HIAA), a well-established biomarker of suicidal behavior.
2. Experimental procedures

2.1. Study setting

Participants were recruited at the Karolinska University Hospital among the patients having a clinical follow-up for a suicide attempt. Patients were invited to take part in a study: “Attempted suicide- biomarkers and psychosocial factors” focusing on psychological and biological risk factors of suicidal behavior. Study protocols (Dnr 93–211; Dnr 2013/917-32) were approved by the Regional Ethical Review Board in Stockholm and participants have given their written informed consent prior to study-inclusion.

2.2. Patients

This clinical cohort study comprised of 42 patients, (27 women and 15 men) with a mean age of 39 years (S.D. 13.2, range 18–69). The study population has been described in detail earlier (Asellus et al., 2010).

The inclusion criteria were a recent suicide attempt, a fair command of Swedish language and age of 18 years or older. Exclusion criteria were schizophrenia spectrum psychosis, dementia, mental retardation and intravenous drug-abuse. We defined a suicide attempt as any self-injurious behavior with some intent to cause death. All participants were diagnosed by a trained psychiatrist using the SCID-1 interview according to the DSM-III. Personality disorders were diagnosed by trained clinical psychologists using the SCID-2 interview.

Most patients, 95%, had a current axis I diagnosis; out of those 78% met criteria for a mood disorder, 7% had an adjustment disorder and 5% anxiety disorder. One patient had a primary substance related disorder and one patient an unspecified psychiatric disorder (not psychotic). Prevalence for a co-morbid personality disorder was 37% and for substance abuse disorders (mainly alcohol dependence) 19%.

2.3. Assessments

For classification of violent and non-violent suicide attempts, we used a dichotimization that has shown relevance concerning biological differences. Self-poisoning and cuts in one wrist were considered as non-violent suicide attempts, while all other, like, attempted drowning, shooting, gassing or hanging were considered to be violent (Träskman et al., 1981).

For assessment of severity of suicidal behavior, we used Beck's Suicide Intent Scale (SIS) and the Freeman Scale (Intent-to-Die Scale). SIS Item 18 was used to assess repeated suicide attempts: 1) none, 2) one or two, and 3) three or more earlier suicide attempts (Beck et al., 1974).

The Freeman scale consists of two parts, Reversibility and Interruption Probability. The Reversibility takes into account the type of drug and the quantity used and the extent of self-injury inflicted. A high score indicates a low reversibility of the suicide attempt method, such as shooting or hanging, ie a more serious suicide attempt with high risk of death, while a low score indicates that the method is reversible and suicide death less likely. The second part of the Freeman scales measures the probability of interruption by others, where high scores indicate that interruption is very unlikely. Both categories are coded on a 1–5 graded scale and the range of scores on total Freeman scale is between 2 and 10 (Freeman et al., 1974). The two scales were applied to a large sample of suicide attempt and suicide death cases showing very good discriminating validity (Freeman et al., 1974). The scales capture important aspects of suicide intent and have been validated using Beck Suicide Intent Scale (Jokinen et al., 2012). Interrater reliability of reversibility of method rating was 0.97 and 0.80 for interruption probability (Freeman et al., 1974).

2.4. Cerebrospinal fluid apolipoprotein E and 5-HIAA analysis

According to the study protocol, there was a washout period of at least 6 days after intoxication. All lumbar punctures were performed in the morning, between 8 and 9 a.m. Patients were fasting in bed since midnight and patients were in a seated position. The needle was inserted between vertebrae IV and V. 12 ml of cerebrospinal fluid was drawn from each patient and the samples were immediately centrifuged and stored at −80°C. For analysis of CSF 5-HIAA mass fragmentography (GC-MS) was used, according to methods developed by Bertilsson. The variation coefficient of the analytical method has been found to be less than 5% (Bertilsson, 1981). Mean time between suicide attempt and lumbar puncture was 23 days (the median 18 days, range 6–65 days).

Apolipoprotein E in CSF was measured using immunonephelometry in BNProSpec Dade Behring. In immunonephelometry ApoE forms complexes with specific antibodies and the instrument measures intensity of the light which is spread by the immunocomplexes (Weisweiler and Schwandt, 1983). Measurements are done according to “fixed-time”-principal. After the adding of antiserum, measurements of light-intensity are recorded at 7.5 s and at 6 min. Level of ApoE in the solution was calculated as difference in light intensity by the use of a calibration-curve. The measurements are done for two intervals, the first after a 1:5 dilution, in which concentrations of 0.01–0.19 g/L (10–190 mg/L) were measured, and the second after a 1:20 dilution in which concentrations of 0.04–0.76 g/L (40 – 760 mg/L) were measured. All results are given as mg/L, without decimals.

2.5. Data analysis

Initial analyses were carried out to evaluate the skewness and kurtosis of the distributions with the Shapiro-Wilk test. One patient was identified both as a univariate and multivariate outlier using Mahalanobis distance. The exclusion of this individual affected the results, and was therefore excluded in all analyses. In continuous variables, group differences were analysed using Student’s t-test. Tests of parametric correlations were performed using Pearson’s r. Fisher’s exact test was used for crosstabulations. The alpha was set at 0.05. The Statistical Package JMP 11.2 Software, SAS Institute Inc., Cary, NC, USA, was used for all statistical analyses.

3. Results

3.1. Characteristics of suicide attempts

Information on earlier suicide attempt(s) from the Suicide Intent Scale could be obtained for 40 suicide attempters. Seventeen patients (42.5%) made their first suicide attempt at the study inclusion, 12 patients (30%) had one or two earlier suicide attempts, and 11 patients (27.5%) reported 3 or more earlier suicide attempts. 23 out of the 40 patients (57.5%) were thus classified as repeaters. The repeater status was not significantly related to age or gender.

Nine patients (23%) had used a violent suicide attempt method (index attempt at inclusion to the study). Violent suicide attempt methods were following: one cutting (not wrist), two hangings, four asphyxiation (gas), and two patients used combination of two violent methods (other than combination of intoxication and wrist cut). There was a trend for first-time suicide attempters to choose more often a violent suicide attempt method (p = 0.10). The mean total score in Freeman scale was 5.88 (S.D. 1.5, range 3–9), the mean Freeman reversibility score was 2.83 (S.D. 0.8, range 1–5) and the mean Freeman Interruption Probability score was 3.05 (S.D. 1, range 1–5).

3.2. CSF apolipoprotein E levels in suicide attempters

The mean CSF ApoE level in suicide attempters was 3.27 mg/l, (n = 41, SD = 1.05, range 1.35–5.21, median 3.33 mg/l). The mean CSF
3.3. CSF apolipoprotein E levels and characteristics of suicidal behavior in suicide attempters

CSF ApoE levels showed a significant negative correlation with Freeman Reversibility scale (r = −0.31, p = 0.049) but not with Freeman Interruption Probability (r = −0.12, p = 0.27), Fig. 1.

Suicide attempters with a violent suicide attempt showed a trend for lower CSF ApoE level (mean = 2.79 mg/l, SD = 1.18, range 1.54–3.52, median 2.79 mg/l, n = 9) compared to suicide attempters with a non-violent method (mean = 3.41 mg/l, SD = 1.10, range 1.35–5.21, median 2.42 mg/l, n = 32) (p = 0.09; Cohen’s d = 0.74).

Patients with at least one earlier suicide attempt (repeaters) showed a trend for higher CSF ApoE level (mean = 3.49 mg/l, SD = 1.10, range 1.54–5.21, median 3.43 mg/l, n = 23), compared to suicide attempters debuting with suicidal behavior at inclusion in the study (mean = 2.91 mg/l, SD = 0.90, range 1.35–4.78, median 2.81 mg/l, n = 17) (p = 0.08).

The correlation between CSF ApoE and 5-HIAA was not significant (r = 0.079, p = 0.46).

4. Discussion

In this study, we report for the first time that suicide attempters who used a more lethal, irreversible suicide attempt method had lower levels of CSF ApoE.

Severity of suicidal behavior is often defined as the lethality of the suicide attempt in combination with the intent to die (Silverman et al., 2007). Given that suicidal behavior is a complex and heterogeneous phenotype, we explored three clinical aspects of severity: a violent, potentially irreversible method of index suicide attempt, suicide intent and temporal severity. There was a trend for first-time suicide attempters to choose more often a violent suicide attempt method compared to repeaters in this study. Suicide attempters using violent methods generally have higher levels of aggression and impulsive behavior, report more adult interpersonal violence and have a higher suicide risk (Jokinen et al., 2010; Stenbacka and Jokinen, 2015). There are earlier findings of an association between low cholesterol and violent suicide and suicide attempt method (Alvarez et al., 2000; Lalicovic et al., 2007); while no earlier studies have reported association between lower levels of ApoE and the choice of more irreversible suicide attempt method. Whether CSF ApoE is a trait or state marker of violent suicide attempt remains to be elucidated.

We have reported that patients, in an independent cohort, with at least one earlier suicide attempt had significantly higher plasma ApoE levels compared to suicide attempters debuting with suicidal behavior. Indicating that plasma ApoE levels may be related to temporal aspects of severity of suicidal behavior (Asellus et al., 2016). It is, however, worth noting that the correlation between levels of ApoE in plasma and in CSF is rather weak (Toledo et al., 2014).

Earlier research of ApoE in CSF has mostly focused on neurodegenerative illnesses or brain trauma. Interesting aspects of ApoE in relation to psychiatric and cognitive illnesses are, however, the role of ApoE in modulation of the inflammatory response and its role in protecting the brain from oxidative injuries (Mahley and Huang, 2012). The finding of low CSF ApoE in relation to seemingly more irreversible suicide attempts may be related to effects similar to those observed after brain trauma (Kay et al., 2003). However, the mean washout time of about three weeks between suicide attempt and lumbar puncture lowers the possibility that lower level of CSF ApoE would result from hypoxic brain damage or substances.

There was no significant correlation between CSF 5-HIAA and CSF ApoE. This is in accordance to an earlier study (Sjögren et al., 2002). Prospective studies of suicide completion and the serotonergic system quite uniformly report that low CSF 5-HIAA levels and a history of attempting suicide predict suicide.

5. Strengths and limitations

The strengths of this study include a well-characterized medication-free cohort of suicide attempters and well-standardized ApoE measurements in CSF. There are also several limitations in this study, such as a relatively small sample size letting us to detect only large effects and the lack of healthy controls.

6. Conclusion

ApoE in CSF may be associated with irreversibility of suicide attempts. This implies the need to replicate these findings in larger samples, especially given the paucity of findings on ApoE and suicidality.

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References

suicide attempters. Psychiatry Res. 95 (2), 103–108.