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APOE Alleles as Predictors of Long-Term Cognitive Outcome after Ischemic Stroke

Degree Project in Medicine

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Abstract

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BACKGROUND: Cognitive impairment after ischemic stroke has a substantial impact on quality of life. Apolipoprotein E (*APOE*) alleles have been shown to predict cognitive decline in the general population, but there is a lack of knowledge about whether they also predict post-stroke cognitive impairment.

AIM: To test the hypothesis that apolipoprotein E (*APOE*) alleles predict post-stroke cognitive impairment, and if so, to understand more about how these associations are affected by possible mediating factors.

METHODS: The study comprised participants from the Sahlgrenska Academy Study on Ischemic Stroke, which consecutively recruited patients with acute ischemic stroke aged 18-69 at stroke units. They were thoroughly characterized with respect to cardiovascular risk factors and stroke etiology at baseline, and blood was collected for determination of *APOE* alleles, serum levels of neurofilament light (NfL) and blood lipids, including high-density lipoprotein (HDL). At a 7-year follow-up, 427 patients underwent cognitive testing by the Barrow Neurological Institute Screen for higher cerebral functions (BNIS).

RESULTS: *APOE* ε4 carriers had lower cognitive function as assessed by BNIS seven years after stroke compared to ε2 carriers (ANOVA MD = 2.6, p = 0.024). This association remained after adjustment for age, stroke severity, education, diabetes mellitus, and hyperlipidemia (linear regression β = 2.2, p = 0.016). After adding interaction terms to the models, there were significant differences between *APOE* allele groups in the association between NfL levels and BNIS score as well as in the association between HDL levels and BNIS score.

CONCLUSIONS: Among young and middle-aged ischemic stroke survivors, *APOE* ε4 carriers have a higher risk of long-term cognitive impairment compared to ε2 carriers.

KEYWORDS: Apolipoprotein E, ischemic stroke, cognitive impairment, risk factors

Introduction

Stroke

Definition of stroke

A stroke arises when blood supply to a brain region is restricted due to a vascular event, resulting in hypoxia and tissue damage. This is outwardly observed as a loss of function depending on the area affected, for example a loss of motor function or language capabilities. The vascular event may be either a hemorrhage, causing hemorrhagic stroke, or an obstruction of the vessel, causing ischemic stroke. Stroke is defined by the World Health Organization as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin" [1]. This definition has existed since the 1970s and is still widely used in clinical practice. It has however been challenged on the grounds that it does not include neuroimaging results, which is central to the diagnosing of stroke, and that brain tissue can suffer permanent damage much sooner than 24 hours [2].

Trends in the burden of stroke

Disability-adjusted life years (DALYs) are defined as the sum of years of life lost (YLL) and years of life lived with disability (YLD) due to a disease, and measures the burden of a disease on society [3]. One DALY is roughly equivalent to a year of life not lived in full health. The three diseases that cause the most DALYs in adults are in descending order: neonatal disorders, ischemic heart disease and stroke [4]. Globally, the overall age-adjusted incidence of stroke has decreased in recent years [5, 6]. However, the population overall as well as stroke survivors live longer, resulting in an increasing number of people living with the consequences of stroke [5]. In contrast to patients of all ages, the incidence among younger patients seems to be increasing [7, 8]. Only every third stroke now affects a person above 70

years of age [5]. Thus, a growing number of young people will live long lives with disability as a result of stroke.

In Sweden and other high-income countries ischemic stroke comprises 85 % of the total number of stroke cases, and it is also the most prevalent form of stroke across the globe [6, 9]. Since 1990 the worldwide prevalence of ischemic stroke among people aged 20-64 years has grown significantly, and in 2013 that prevalence was 7.3 million [5]. The total number of DALYs caused by all stroke grew by 16 % from 2007 to 2017, and the number caused by ischemic stroke specifically grew by 25 % in the same time period [4]. With this in mind, it seems that ischemic stroke is playing a progressively greater role in causing disability and death compared to hemorrhagic stroke.

Classification of ischemic stroke

Trial of Org. 10172 in Acute Stroke Treatment (TOAST) is the most commonly used classification method of ischemic stroke and divides ischemic stroke according to pathophysiological mechanism into large-vessel disease (LVD), small-vessel disease (SVD), cardioembolic stroke (CE), other determined etiology and undetermined etiology [10, 11].

Large-vessel disease constitutes about 20 % of all cases of ischemic stroke [12]. Larger precerebral and cerebral arteries are occluded, and areas in the brain stem, cerebellum, subcortex and cortex are damaged [6]. The cause of LVD is atherosclerosis [11]. Cardioembolic stroke causes around 20 % of all ischemic strokes [12]. In this subtype, an embolus originating from the heart or aortic arch stops blood flow in an artery of the brain [6]. Atrial fibrillation is often the cause of the formation of such an embolus [11].

Small-vessel disease is the etiology in 20-25 % of ischemic stroke cases [12, 13]. Blockage of small perforating arteries branching off from the circle of Willis or the basilar artery is the cause of SVD [14]. The thalamus, the internal striatum, the deep white matter and certain regions of the brain stem are the areas usually affected [6]. Intimal layer thickening,

arterial wall fibrosis, microatheroma (microscopic fatty deposits in arterial walls) and lipohyalinosis (wall degeneration and subsequent vessel narrowing) have been proposed as mechanisms, however the underlying etiological processes have yet to be determined with certainty [15].

Post-stroke cognitive impairment

Some patients fully recover after stroke, but certainly not all. For many, surviving a stroke means living with varying degrees of impairments for the rest of their life. While motor impairments may be more apparent and well-known, cognitive impairment is also common and has a significant impact on quality of life after stroke [6]. For example, in one study of ischemic stroke survivors younger than 50 years, about half of the participants had some form of cognitive impairment 11 years after their stroke [16]. Cognition broadly refers to the intake, processing and output of information in the brain, and encompasses attention, memory and speech production.

The knowledge about effective interventions against post-stroke cognitive impairment is still evolving. Strategies which help the stroke survivor compensate for cognitive deficits as well as possible pharmacological treatments have been described [17]. Psychological interventions may also be beneficial [18].

Cognitive screening tests

A number of tests are available for assessment of cognitive function. A battery of different neuropsychological tests is commonly used to give an in-depth assessment of different domains of cognitive function [6]. However, such a battery demands a great deal of time and effort for both the examiner and the patient [6]. Screening tests have been designed to fill the need for relatively quick, easy-to-administer tests with adequate sensitivity and specificity to detect those with possible cognitive impairment, who may need further testing [6]. Among the most widely used is the Mini Mental State Examination (MMSE) [19]. MMSE was

primarily devised to detect dementia, and is not finely tuned to detect more subtle cognitive changes which follow stroke, especially over time [20]. Another disadvantage to the test is its ceiling effect, i.e. a large portion of test-takers tend to receive the maximum score or close to it, which makes it difficult to detect higher levels of performance or improvement [21]. The Montreal Cognitive Assessment (MoCA), is more sensitive and offers testing of attention and executive function, which MMSE does not [22]. MoCA has therefore become progressively more popular as a tool for cognitive screening after stroke in recent years. However, MoCA has also been criticized for its low specificity, and that the cut-off points used lead to underestimation of post-stroke cognitive deficits [23].

The Barrow Neurological Institute Screen for higher cerebral functions (BNIS) is a test constructed for quick evaluation of a variety of cognitive domains [24]. The different subscores are summed to give a total score which ranges from 0 to 50 points as detailed in **Table 1**, where a higher score corresponds to better cognitive performance [6]. Before testing, participants must pass a pre-screen test to assess whether they are capable of performing the test [6]. BNIS has been demonstrated to be valid for screening of deficits in cognition in a stroke patient population [25]. Its utility in detecting cognitive impairment in the long-term follow-up after stroke has also been shown [26]. BNIS covers several cognitive subdomains that are not part of MoCA, including a more in-depth evaluation of language (paraphasia, dysarthria, comprehension, reading, writing, spelling), visuospatial abilities (visual object recognition, scanning and sequencing as well as recognition and copying of patterns) and affect (expression, perception and control of affect) [21, 24]. Subdomains measured by MoCA, but not BNIS, are fewer, and the most notable is abstraction capability [21, 24]. The subdomains tested by BNIS but not by MoCA are commonly affected by stroke, which speaks in favor of BNIS for cognitive screening after stroke.

Table 1. Description of the items of the Barrow Neurological Institute Screen for higher cerebral functions (BNIS), adapted with permission from Pedersén [6].

BNIS items	Score	Subscale score
Pre-screening		
Level of consciousness	3	
Basal communication	3	
Cooperation	3	9
Speech and language		
Fluency	1	
Paraphasia	1	
Dysarthria	1	
Comprehension	2	
Naming	1	
Repetition	2	
Reading	1	
Writing – sentence copying	1	
Writing – dictamen	1	
Spelling – irregular	1	
Spelling – phonetic	1	
Arithmetic – number/symbol alexia	1	
Arithmetic – dyscalculia	1	15
Orientation		
Left-right orientation	1	
Place orientation	1	
Time orientation	1	3
Attention/concentration		
Arithmetic memory/concentration	1	
Digits – forward	1	
Digits – backward	1	3
Visuospatial and visual problem-solving		
Visual object recognition	1	
Constructional praxis dominant hand	1	
Constructional praxis non-dominant hand	1	
Visual scanning	2	
Visual sequencing	1	
Pattern copying	1	
Pattern recognition	1	8
Memory		
Number/symbol test	4	
Delayed recall	3	7
Affect		
Affect expression	1	
Affect perception	1	
Affect control	1	
Spontaneous affect	1	4
Awareness		
Awareness vs. performance	1	1
Total score	50	

Factors of importance for the risk of cognitive impairment

Factors of importance in stroke survivor populations

Clinical factors

Few studies have looked at the factors of importance for the risk of cognitive impairment in pure stroke survivor populations. Three studies that also considered genetic risk found clinical variables of importance for the risk of post-stroke cognitive impairment [27-29]. Poor cognitive performance at baseline, depressive symptoms as well as the presence of cardiovascular risk factors were associated to post-stroke dementia in an 8-year follow-up study [29]. Two studies with a shorter follow-up (3 weeks and 13 months) found pre-stroke cognitive impairment and neurological impairment to be associated with increased risk of having a cognitive score below a cut-off value after stroke [27, 28]. Anterior stroke syndrome was also identified as a risk factor in the former study [27], and previous stroke in the latter [28]. One systematic review and one meta-analysis have both concluded that physical activity is beneficial for post-stroke cognitive function [30, 31]. Several studies have also reported that higher education level is associated to better cognitive performance after stroke [32-34].

Genetics

The most well-known the genetic factor that influences cognitive function is the gene encoding for apolipoprotein E (*APOE*), which will be expanded upon shortly. In order to provide some background for this, the following section will give an overview of some fundamental concepts in genetics.

The human genome is arranged in 23 pairs of chromosomes, which are made up of rigidly packed deoxyribonucleic acid (DNA). DNA is a polymer made up of nucleotide monomers, which in turn consist of a nucleobase, a ribose and a phosphate group. Nucleobases come in four variants: adenine (A), thymine (T), cytosine (C) and guanine (G). A single-nucleotide polymorphism (SNP) is a nucleotide at a specific position in the genome which is different in more than 1 % of the population. Alleles are the possible nucleotide variants of an

SNP. Since chromosomes come in pairs, the pair of alleles (the genotype) may be either the same (homozygous) or different (heterozygous). During meiosis, a pair of chromosomes swap portions of their DNA with each other in a process called recombination. SNPs which lie close together in one region of the chromosome tend to stay together during this process. These regions are termed haplotypes and the SNPs within a haplotype are described as being linked.

The genotype of an SNP can be determined by two main types of methods: direct genotyping and imputation. Direct genotyping is generally resource-intensive and is typically done only for a smaller number of SNPs of interest. Imputation on the other hand makes use of haplotypes. It does this by first determining the genotype of a number of SNPs spread throughout the genome. Because the SNPs in a haplotype are linked, the probability of genotypes of other SNPs in the same haplotype can be inferred using statistical methods. It uses the information from the known SNPs in the genome of interest, and a reference genome that has been genotyped for a larger number of SNPs. The method returns the dosage of the rare allele, ranging from 0 to 2, where the integers mean for example AA-AG-GG where G is the rare allele. Non-integer values reflect the innate uncertainty that comes with the method.

Apolipoprotein E

In the body, hydrophobic compounds e.g. lipids and cholesterol need to be transported in aqueous solutions such as blood and cerebrospinal fluid. This task is performed by apolipoproteins, which are hydrophilic and form bonds with the lipids, which results in lipoproteins. Examples of lipoproteins are low-density lipoprotein (LDL) and high-density lipoprotein (HDL). There are many classes of apolipoproteins, including ApoA, ApoB and ApoE. In the central nervous system, ApoE is the most common apolipoprotein [35]. The *APOE* gene has three major alleles, ε2, ε3 and ε4. Worldwide, the most common allele is ε3 (allele frequency 78%), followed by ε4 (14%) with ε2 being the least common (8%) [36].

The *APOE* genotype is determined by two SNPs, rs7412 and rs429358, which can take the forms of CC, CT and TT. The combinations of genotypes in these two SNPs determine the *APOE* genotype. Presence of the T allele (CT and TT) in rs7412 corresponds to the ε2 allele and presence of the C allele (CT and CC) in rs429358 corresponds to the ε4 allele. The presence of C in rs7412 (CT and CC) together with the presence of T (CT and TT) in rs429358 corresponds to the ε3 allele, except for the fact that having CT in both SNPs corresponds to the genotype ε4/ε2.

The ε4 allele is a well-established as a risk factor of Alzheimer's disease (AD), while the ε2 allele has a protective association to AD [36]. In the brain, apolipoprotein E binds to amyloid β (Aβ) and affects how neurons amass and rid themselves of Aβ [36]. The different *APOE* alleles correspond to structural differences in the ApoE protein, the E2, E3 and E4 isoforms, which have different affinities for Aβ. The E4 isoform does not clear Aβ as effectively as E3, and deposits Aβ to a higher degree [36]. Exactly how Aβ then is toxic to the neurons is unclear, but it has been suggested that Aβ causes disruption of the cellular membrane function, leading to a change in ion concentrations and impaired cellular function [37]. This would presumably lead to the destruction of the neuron and its axons. Furthermore, the ε4 allele is associated with cardiovascular disease in general, for example vascular dementia [35], which of course contributes to cognitive decline. There are also a differences between *APOE* alleles in the prevalence of hyperlipidemia, a risk factor of cardiovascular disease, as well as lipid and lipoprotein levels [38].

In pure stroke survivor populations, few studies on associations between *APOE* and cognitive outcomes have been conducted [27-29, 39-42], and those that exist often have a low number of participants and generally study older patients. Our group recently found an association between ε4 carriage and younger age at stroke onset [43]. Thus, there is a need for larger studies on *APOE* alleles and long-term cognitive impairment, especially among the young and

middle-aged. Knowledge about the effects of the ε2 allele on post-stroke cognition is particularly scarce. Another interesting research topic that is relatively unexplored is whether there is an interaction between lifestyle factors described above, such as physical activity, and genetic risk with respect to cognition among stroke survivors.

Factors of importance in the general population

Clinical factors

Since the important clinical factors for cognitive impairment in stroke survivor populations have not been extensively studied, it is relevant to look at the knowledge about these factors in the general population. A recent study that pooled data from 20 population-based cohorts from 15 countries found that higher age, history of stroke, depression, diabetes mellitus and current smoking were all associated with worse performance on cognitive tests [44]. In addition, age and diabetes were linked with faster cognitive decline [44]. Furthermore, higher levels of education and physical activity were associated with better performance on cognitive tests [44].

Apolipoprotein E

In studies based on the general population, the association between *APOE* alleles, stroke and cognitive decline has been investigated. In the 20-cohort study mentioned above, a correlation between *APOE* ε4 carriage and poorer performance on cognitive tests was found, and ε4 carriage was also associated to faster cognitive decline [44]. Three other population-based studies that included stroke as a variable have found an association between ε4 carriage and dementia or cognitive score decline [45-47]. None of these three reported that stroke modified the association between ε4 carriage and cognition. Surprisingly, another population-based study found a difference in cognitive decline after incident stroke in the *non*-ε4 carrier group, but not among ε4 carriers [48]. Two other population-based studies did however find some form of positive interaction (modified relationship) between ε4, stroke and the risk of incident dementia [49, 50], i.e. the association between stroke and the risk of dementia was

stronger if the ε4 allele was present. Conversely, two additional population-based studies that looked at patients both with and without stroke suggest that ε4-carrying stroke survivors have a lower cognitive decline or risk thereof compared to non-ε4 carrying stroke survivors [51, 52].

Neurofilament light chain

The cytoskeleton of axons consists of a variety of structural proteins, among which neurofilaments are the predominant kind. Neurofilaments are specific to neurons and consist of several subunits, one of which is neurofilament light chain (NfL) [53]. When axons are damaged or destroyed, neurofilaments spill out of the cell and eventually reach the cerebrospinal fluid (CSF) and the blood stream [54]. The concentration of NfL could previously only be reliably measured in CSF because of the low concentrations in the blood, which necessitated lumbar puncture. In recent years, a single-molecule immunoassay (SiMoA) technology has been developed which allows for sensitive measurement of serum NfL [55, 56]. Hence, serum NfL levels can now be used as a biomarker for axonal damage and the progression of neurodegenerative and cerebrovascular diseases in studies where lumbar puncture is not a feasible option.

Underlying mechanisms

The processes through which risk factors and protective factors influence the brain's cognitive function are only partially understood. With age comes brain atrophy, and loss of both grey and white matter volume. A portion of this is due to neuronal death, but much can also be attributed to the changing structure of the neurons, which includes loss of myelination, axons, dendrites and synapses [57]. Type 2 diabetes is connected to an elevated risk of cerebral small vessel disease, and Alzheimer's disease may be more common among patients with diabetes mellitus [58]. Depression has been proposed to lie on a continuum with mild cognitive impairment and dementia, in that they share biological mechanisms such as

neurodegeneration [59]. Physical exercise improves blood flow to the brain, increasing the number of synapses which may provide cognitive reserves that protect against cognitive decline [60]. Education may also increase such cognitive reserves [61].

Relevance of the study

Disability caused by stroke in general, and ischemic stroke in particular, imposes a heavy burden on both society and the affected individual. The younger the affected individual is, the heavier is the burden, and stroke is increasing among the young. Cognitive impairment in particular presents many challenges for the patient. The clinical risk factors of cognitive dysfunction are known, but they are not fully able to predict why certain individuals do cognitively worse than others. There are however biomarkers which could be useful in predicting cognitive outcome, *APOE* alleles being one example. Furthermore, the mechanisms behind cognitive impairment are not fully understood and warrant further exploration. If post-stroke cognitive impairment can be more accurately forecast, rehabilitation can be more effectively focused on those who need it the most.

Aim

The main aim of this study is to investigate whether *APOE* alleles predict long-term cognitive outcome after ischemic stroke in young and middle-aged patients. The specific research questions are defined as follows:

Primary research question: Is there an association between *APOE* alleles and cognitive abilities in the long term after ischemic stroke?

Secondary research questions:

If that is the case, can we understand more about the underlying biological mechanisms by investigating associations between *APOE* alleles and cardiovascular risk factors for cognitive impairment, as well as the neuronal damage biomarker NfL?

How well does imputation of SNP array genotype data determine *APOE* genotypes and alleles compared to direct genotyping?

Is there an association between *APOE* alleles and serum levels of NfL after ischemic stroke?

For the first question, we hypothesized that the greatest difference between *APOE* allele groups in post-stroke cognitive function lies between carriers of the ε4 allele and carriers of the ε2 allele.

Material and Methods

Sahlgrenska Academy Study on Ischemic Stroke

This study is based on data that had already been collected as part of the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), which was a longitudinal observational study of the outcome after ischemic stroke, with a focus on underlying genetic factors and family history of stroke [62]. The term "participant" (unless further specified) will hereafter be used to refer to any individual who participated in the study, i.e. both cases and controls.

Inclusion

SAHLSIS included individuals who were diagnosed with acute ischemic stroke between the years 1998-2017 [6], henceforth referred to as cases. Stroke diagnosis was defined as a sudden onset of symptoms consistent with a cerebrovascular lesion together with the absence of cerebral hemorrhage on magnetic resonance imaging (MRI) or computer tomography (CT). The cases originated from West Sweden and were between 18-69 years old at inclusion. Furthermore, cases were excluded if they belonged to a non-Caucasian ethnicity, if they had human immunodeficiency virus (HIV), advanced-stage cancer or infectious hepatitis, or if a cause of the symptoms other than stroke was found.

Four stroke units in the area took part in the first phase of the study: two at Sahlgrenska University Hospital (Sahlgrenska and Östra), one at Södra Älvsborg Hospital in Borås and

one at Skaraborg Hospital in Skövde. This first phase comprised 600 cases and lasted between 1998 and 2003. There was also a control group of 600 participants without cardiovascular disease matched by age, sex and geographical area to the cases, henceforth referred to as controls. Following the first phase, cases were continuously recruited to the study at the stroke unit at Sahlgrenska until December 2017. In total, 1590 stroke patients participated.

Baseline characteristics

The etiological stroke subtype was determined using the classification system Trial of Org 10172 in Acute Stroke Treatment (TOAST). Stroke severity, i.e. degree of neurological deficit, was assessed repeatedly using the Scandinavian Stroke Scale (SSS) at inclusion, and the worst score was used. Information about cardiovascular risk factors such as hypertension, hyperlipidemia, diabetes mellitus, atrial fibrillation, physical activity and smoking status were gathered via questionnaires, as well as by pre-defined criteria from medical records. This was done at inclusion, after three months and after seven years. The criteria used to define these conditions have been described previously [63].

Blood sampling

Blood was sampled at all three timepoints for cases and at inclusion for controls. Serum was isolated and aliquoted. Whole blood and serum was then frozen and biobanked at -80 ºC, at the Department of Clinical Genetics, Sahlgrenska University Hospital.

Follow-up and outcomes

A follow-up visit to a physician took place after three months. Seven years after their inclusion, surviving cases who entered the study at Sahlgrenska before January 26, 2009 received invitations to follow-up visits with a nurse and a physician. **Figure 1** displays the study population and non-respondents for this seven-year follow-up. Data on recurrent strokes during follow-up was collected via questionnaires and national registers. Registered events were then verified by examination of medical records. Neurological deficits were assessed again

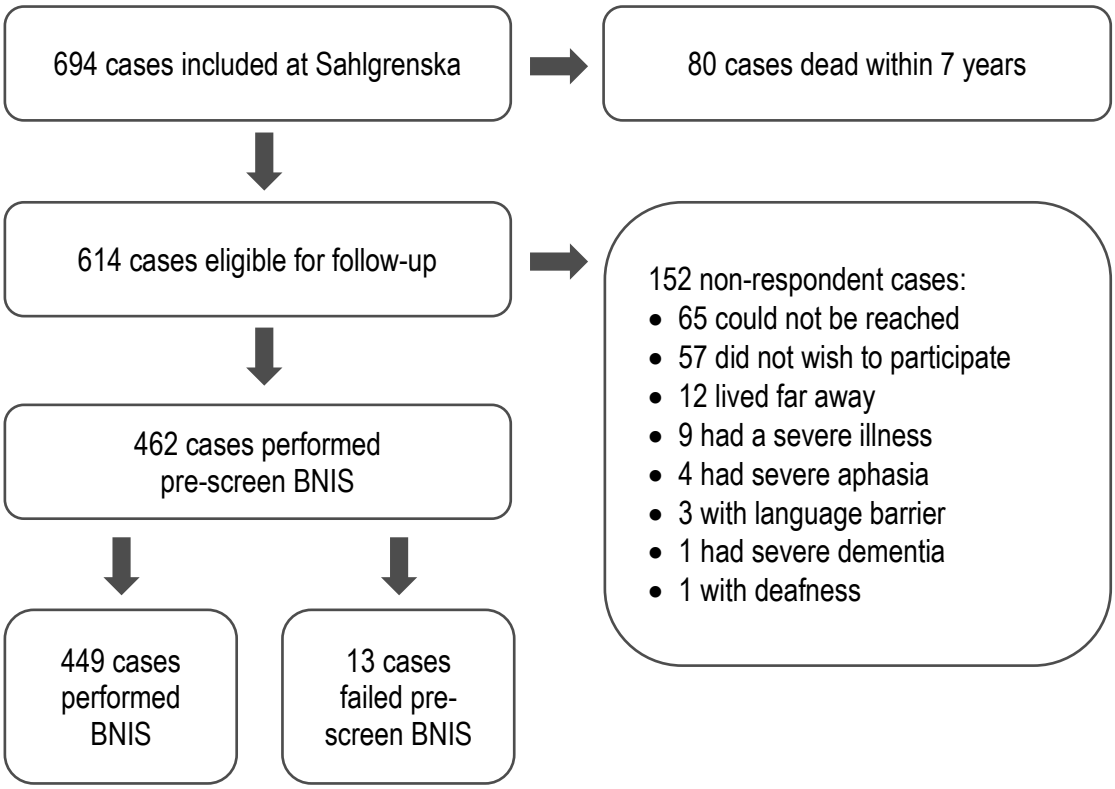

Figure 1. Flowchart of the 7-year follow-up study population and the recruitment process. BNIS, Barrow Neurological Institute Screen for higher cerebral functions. Sahlgrenska, the stroke unit at Sahlgrenska University Hospital/Sahlgrenska.

using SSS after three months and using the National Institutes of Health Stroke Scale (NIHSS) after seven years. Information about education level and physical activity was gathered via questionnaires. Serum levels of NfL were also considered as an outcome variable in certain analyses (see below).

At the seven-year visit, cognitive function was assessed by validated cognitive tests, including the Barrow Neurological Institute Screen for higher cerebral functions (BNIS), which is described more in-depth in the Introduction (page 8). Those who passed the pre-screen and performed BNIS will hereafter be referred to as BNIS participants, and all those included who came into question for the follow-up but declined participation or did not pass the pre-screen will be referred to as BNIS non-participants.

Dementia or Alzheimer's disease was not explicitly asked about in the questionnaires, although an open question about whether the BNIS participants had any severe diseases was included. However, one participant (**Figure 1**) was excluded because of severe dementia.

Analysis of NfL

Serum NfL levels were measured using single-molecule assay (SiMoA) technology as described in detail elsewhere [62]. This was done for phase 1 cases and for controls.

Genotyping

This study also included an evaluation of how well imputed genotypes from SNP arrays can be used to determine *APOE* genotypes. This evaluation included all participants in SAHLSIS, i.e. not only the cases included in the present study on cognition, but also the healthy controls. First, *APOE* genotypes were determined by direct genotyping of the SNPs rs7412 and rs429358 by means of allelic discrimination technology [64], which is based on a polymerase chain reaction (PCR) followed by detection of fluorescence from dye-marked probes that bind specifically to an SNP allele [65]. Then, we collected genotypes for these SNPs from SNP array data as described [43]. In brief, a large number of SNPs across the genome was genotyped on SNP arrays, and genotypes for additional SNPs were then inferred by imputation to the 1000 Genomes Phase 3 [43]. These imputed genotypes for rs7412 and rs429358 were subsequently used to calculate the corresponding *APOE* genotypes.

Variables

The acute and 3-month SSS were converted into the National Institutes of Health Stroke Scale (NIHSS) using a validated formula [66] because of the wider international usage of the NIHSS. Serum NfL levels were logarithmized to base 10 to be more in line with the normal distribution and to facilitate statistical analysis. Education level was recoded into three groups: elementary (did not complete or completed elementary school), secondary (completed upper secondary school, trade school or college) and university (completed university or

research education). Tough/competitive physical activity (sports participation several times a week regularly) and regular physical activity (sports participation or heavy outdoor work once every week for at least 2 hours on average) was grouped as high physical activity, also resulting in three groups, together with moderate physical activity (walking, biking or light outdoor work for at least 4 hours per week on average) and sedentary physical activity (spending one's time mostly sitting for example reading or watching TV).

The imputed minor allele dosages of rs7412(C) and rs429358(T) were rounded to the nearest integer, and a new combined variable was calculated for the corresponding genotype. Both imputed and directly genotyped genotypes were subsequently grouped into four groups: ε2 carriers (ε2/ε2 and ε3/ε2), ε3/ε3, ε4/ε2 and ε4 carriers (ε4/ε3 and ε4/ε4). This was done in order to have sufficient group sizes. The group with ε4/ε2 was excluded from analyses because it contained both high and low risk alleles, which was deemed to not fit into any of the groups. After the agreement analysis (see below), for cases for which there were imputed data but not directly genotyped data, imputed data were used instead.

Statistical methods

To test the concordance of imputation and direct genotyping, an agreement analysis was performed for the two methods and Cohen's kappa was calculated. Univariate analyses using analysis of variance (ANOVA), Kruskal-Wallis test and Fischer's exact test were performed for differences between the BNIS participants and BNIS non-participants at baseline, as well as between the *APOE* allele groups at baseline and at seven years after stroke. Chi-square test on allele frequencies for departure from Hardy-Weinberg equilibrium was performed. ANOVA and multiple linear regressions were carried out with serum NfL levels and BNIS score as outcome variables. The greatest difference in BNIS score was hypothesized to lie between ε2 carriers and ε4 carriers, and how ε4 carriers fared compared to the largest group of ε3 homozygotes was also of interest, leading to the choice of ε4 carriers as the

reference category for *APOE* alleles. Regressions of BNIS score were performed unadjusted as well as adjusted for age, education, diabetes mellitus and baseline NIHSS. A sensitivity analysis, where cases with recurrent stroke were excluded, was also performed.

Interaction terms[1] were added in linear regression analyses of BNIS with sex, physical activity, age, serum NfL and high-density lipoprotein (HDL) as predictors. In interaction analyses, continuous variables were centered on their median or mean (i.e. the median or mean was subtracted from all values) to ease the interpretation of regression coefficients for main effects. Mediation by hyperlipidemia as well as blood lipids was analyzed with logistic and linear regressions according to the causal steps approach. All statistical tests were deemed as satisfying their respective assumptions. All analyses were performed using the statistical software SPSS (version 25), except Chi-square test for Hardy-Weinberg equilibrium which was carried out in Microsoft Office 365 Excel (version 18).

Ethics

The Regional Ethics Committee in Gothenburg approved SAHLSIS. All participants were informed about the study and gave written consent for their participation. In those cases where patients were incapacitated or otherwise unable to give consent, their next of kin gave written consent.

Some of the central principles of the Helsinki Declaration, a fundamental ethical framework for biomedical research on humans [67], are that the person who participates in research is entitled to the ability to make choices about their own life, as well as adequate information to make those choices [68]. A similar sentiment exists in the Universal Declaration of

[1] An interaction term in a linear regression equation is the product of two independent variables, and allows for the study of how the association between one independent variable and the dependent variable varies across levels of a second independent variable. If the coefficient for such a term is statistically significantly different from zero, it can be said with confidence that the association between the one independent variable and the dependent variable differs based on the level of the second independent variable.

Human Rights, in that "[e]veryone has the right to life, liberty and security of person" [69]. In line with this, participants in SAHLSIS have been informed about their ability to withdraw from the study in full or to not participate in certain parts. One has to keep in mind however that stroke survivors are an especially vulnerable group, who may not be fully able to exercise these rights because of various post-stroke impairments.

Biobanked information is regulated by the Biobank Law (SFS 2002:297) in Sweden and the storage of personal information is regulated by the General Data Protection Regulation in the European Union [70]. Genetic data connected to a specific person in particular is considered to be highly sensitive personal information [70]. In SAHLSIS, participants have of course been pseudonymized and the authors were blinded to the identities of the participants in order to protect their integrity.

Results

Characteristics of the cohort

Baseline characteristics for BNIS participants and BNIS non-participants are displayed in **Table 2**. Notably, BNIS participants had statistically significantly lower rates of diabetes mellitus and smoking and lower stroke severity (NIHSS) at baseline. **Table 3** shows the baseline characteristics for the BNIS participants according to their *APOE* allele group, excluding those who were missing *APOE* data (n = 2) and those with the ε4/ε2 genotype (n = 20). Hyperlipidemia prevalence was the only variable that showed a statistically significant difference between the allele groups. In **Table 4**, the characteristics at the seven-year follow-up are shown. No statistically significant differences between the groups were found for any of the characteristics, save for BNIS total score (see page 24).

APOE genotypes and alleles

Based on 1,369 individuals (775 cases and 594 controls) for whom genotype data from both imputation and direct genotyping was available, the two methods showed a very high level of

Table 2. Baseline characteristics for BNIS participants and BNIS non-participants (n = 694).

		Missing	BNIS participants	BNIS non-participants	Total	*p* for difference†
			n = 449	n = 245	n = 694	
Age in years, median (IQR)		0	57 (48–63)	59 (50–63)	57 (49–63)	0.10
Men		0	286 (64%)	165 (67%)	451 (65%)	0.36
NIHSS, median (IQR)		5 (0.72%)	2 (1–6)	5 (2–12)	3 (2–8)	1.4E-7**
History of stroke		5 (0.72%)	62 (14%)	32 (13%)	94 (14%)	0.91
Hypertension		4 (0.58%)	257 (57%)	153 (63%)	410 (59%)	0.14
Diabetes mellitus		0	79 (18%)	62 (25%)	141 (20%)	0.018*
BMI in kg/m²		29 (4.2%)	27 (4.5)	26 (4.3)	26 (4.4)	0.37
Hyperlipidemia		45 (6.5%)	291 (68%)	154 (70%)	445 (69%)	0.53
Smoking		9 (1.3%)	151 (34%)	113 (47%)	264 (39%)	9.8E-4**
Physical activity	**Sedentary**	51 (7.3%)	73 (17%)	54 (24%)	127 (20%)	0.097
	Moderate		250 (59%)	122 (55%)	372 (58%)	
	High		99 (23%)	45 (20%)	144 (22%)	

BNIS, Barrow Neurological Institute Screen for higher cerebral functions; NIHSS, National Institutes of Health Stroke Scale; n, number of individuals in the group; IQR, interquartile range; BMI, body mass index; * $p < 0.05$; ** $p < 0.001$; † between BNIS participants and BNIS non-participants. Data are displayed as frequency (percentage) for categorical variables and as mean (standard deviation) for continuous variables unless indicated otherwise. Characteristics were compared between the groups for normally distributed variables using independent samples *t* tests. For non-normally distributed variables, this was done using Mann-Whitney *U* test. For categorical variables, Fisher's exact test was used. The *p* values are not corrected for multiple testing.

agreement for the genotypes ($\kappa = 0.9817$, $p < 0.001$, 95% confidence interval (CI) = [0.9725, 0.9909]). For the grouped *APOE* allele variable, the agreement between the methods was slightly greater ($\kappa = 0.9824$, $p < 0.001$, 95% CI = [0.9732, 0.9916]). In the BNIS study population of 449 individuals, directly genotyped data was available for 443. Of the 6 individuals missing data, imputed data was available for 4, which was added to the variable used.

For the BNIS participants with *APOE* data (n = 447), observed genotype frequencies were ε2/ε2: 3 (0.67%), ε3/ε2: 48 (11%), ε3/ε3: 228 (51%), ε4/ε2: 20 (4.5%), ε4/ε3: 130 (29%) and ε4/ε4: 18 (4.0%). Consequently, allele frequencies were ε2: 37 (8.3%), ε3: 317 (71%) and ε4: 93 (21%). The observed genotype frequencies did not depart from the expected frequencies according to the Hardy-Weinberg equilibrium (Chi-square test p = 0.86). After excluding BNIS participants having the ε4/ε2 genotype (n = 20), the BNIS study population consisted of 3 individuals with imputed data and 424 with directly genotyped data.

Table 3. Baseline characteristics for the *APOE* allele groups among the BNIS participants (n = 427).

		Missing	ε2 carrier	ε3/ε3	ε4 carrier	Total	*p* for difference†
			n = 51	n = 228	n = 148	n = 427	
Age in years, median (IQR)		0	55 (47–61)	57 (50–63)	56 (47–64)	57 (49–63)	0.35
Men		0	31 (61%)	157 (69%)	85 (57%)	273 (64%)	0.071
NIHSS score, median (IQR)		0	2 (1–6)	3 (1–6)	3 (1–6)	3 (1–6)	0.57
History of stroke		0	6 (12%)	36 (16%)	16 (11%)	58 (14%)	0.38
Hypertension		1 (0.23%)	26 (51%)	140 (61%)	82 (56%)	248 (58%)	0.30
Diabetes mellitus		0	9 (18%)	48 (21%)	19 (13%)	76 (18%)	0.12
BMI in kg/m²		11 (2.6%)	26 (4.0)	27 (4.5)	26 (4.6)	27 (4.5)	0.19
Hyperlipidemia		19 (4.4%)	22 (45%)	158 (72%)	102 (73%)	282 (69%)	7.8E-4**
Smoking		4 (0.94%)	19 (37%)	77 (34%)	45 (31%)	141 (33%)	0.72
Physical activity	**Sedentary**	25 (5.9%)	5 (10%)	43 (20%)	22 (16%)	70 (17%)	0.46
	Moderate		33 (69%)	124 (58%)	81 (58%)	238 (59%)	
	High		10 (21%)	48 (22%)	36 (26%)	94 (23%)	

APOE, apolipoprotein E gene; NIHSS, National Institutes of Health Stroke Scale; n, number of individuals; IQR, interquartile range; BMI, body mass index; ** p < 0.001; † between the three *APOE* allele groups. Data are displayed as frequency (percentage) for categorical variables and as mean (standard deviation) for continuous variables unless indicated otherwise. Characteristics were compared between the groups for normally distributed variables using analysis of variance (ANOVA) tests. For non-normally distributed variables, this was done using Kruskal–Wallis test. For categorical variables, Fisher's exact test was used. The *p* values are not corrected for multiple testing.

APOE alleles and BNIS

There was a statistically significant difference in BNIS score between *APOE* groups (ANOVA F(2, 424) = 3.5, p = 0.032), as displayed in **Table 4**. Tukey post hoc testing revealed that there was a statistically significant difference between ε2 carriers and ε4 carriers (mean difference (MD) = 2.6, p = 0.024, 95% CI = [0.28, 5.0]). Differences were not statistically significant between ε2 carriers and ε3 homozygotes (MD = 2.0, p = 0.083, 95% CI = [-0.20, 4.3]) or between ε3 homozygotes and ε4 carriers (MD = 0.59, p = 0.64, 95% CI = [-0.95, 2.1]).

The same analysis stratified by sex showed a statistically significant difference between the *APOE* groups in women (F(2,151) = 3.1, p = 0.049), but not in men (F(2,270) = 1.1, p = 0.34). In a Tukey post hoc analysis for women only, the statistically significant difference was again between ε2 carriers and ε4 carriers (MD = 4.0, p = 0.046, 95% CI = [0.06, 8.0]).

Table 4. Seven-year characteristics for the *APOE* allele groups among the BNIS participants (n = 427).

		Missing	ε2 carrier	ε3/ε3	ε4 carrier	Total	*p* for difference†
			n = 51	n = 228	n = 148	n = 427	
BNIS total score		0	41.1 (5.2)	39.1 (5.9)	38.5 (6.9)	39.1 (6.2)	0.032*
NIHSS score, median (IQR)		16 (3.7%)	0 (0–1)	0 (0–2)	0 (0–2)	0 (0–1)	0.42
HAD depression, median (IQR)		18 (4.2%)	3 (1–6)	3 (1–7)	3 (1–7)	3 (1–7)	0.56
Recurrent stroke		0	4 (7.8%)	35 (15%)	16 (11%)	55 (13%)	0.25
BMI in kg/m²		15 (3.5%)	27 (4.6)	28 (4.6)	28 (4.7)	28 (4.6)	0.37
Smoking		13 (3.0%)	27 (54%)	144 (66%)	93 (64%)	264 (64%)	0.30
Physical activity	**Sedentary**	8 (1.9%)	13 (26%)	64 (29%)	36 (25%)	113 (27%)	0.90
	Moderate		22 (44%)	102 (46%)	69 (47%)	193 (46%)	
	High		15 (30%)	57 (26%)	41 (28%)	113 (27%)	
Education level	**Elementary**	15 (3.5%)	11 (22%)	70 (32%)	39 (27%)	120 (29%)	0.61
	Secondary		22 (45%)	84 (38%)	64 (44%)	170 (41%)	
	University		16 (33%)	65 (30%)	41 (28%)	122 (30%)	

APOE, apolipoprotein E gene; BNIS, Barrow Neurological Institute Screen for higher cerebral functions; NIHSS, National Institutes of Health Stroke Scale; n, number of individuals; IQR, interquartile range; BMI, body mass index; HAD depression, depression subscale of the Hospital Anxiety and Depression scale; * $p < 0.05$; † between the three *APOE* allele groups. Data are displayed as frequency (percentage) for categorical variables and as mean (standard deviation) for continuous variables unless indicated otherwise. Characteristics were compared between the groups for normally distributed variables using analysis of variance (ANOVA) tests. For non-normally distributed variables, this was done using Kruskal–Wallis test. For categorical variables, Fisher's exact test was used. The *p* values are not corrected for multiple testing.

However, linear regression of BNIS score by *APOE* alleles (reference: ε4 carriers), sex (reference: female sex) and age with the *APOE* × sex interaction terms did not yield any statistically significant coefficients for sex ($p > 0.4$) or interaction coefficients (both $p > 0.2$), although the ε2 vs. ε4 term remained statistically significant when adjusting for age ($\beta = 4.0$, $p = 0.011$).

Linear regression was performed for BNIS with age and *APOE* alleles as predictors, subsequently adding education, diabetes mellitus and hyperlipidemia as predictors as seen in the subtables **A**, **B**, **C** and **D** of **Table 5**. Notably, the beta coefficients for ε2 carriage vs. ε4 carriage, age, education and diabetes mellitus remained statistically significant in all four models. Additionally, when adding NIHSS at baseline to the model in **Table 5 D**, the coefficient for ε2 vs. ε4 ($\beta = 2.2$, $p = 0.016$) remained statistically significant. This held true even after excluding those with recurrent stroke ($\beta = 2.0$, $p = 0.036$), and in this subset the ε3 vs. ε4

Table 5. Linear regression of BNIS score by *APOE* alleles and baseline characteristics.

A. By *APOE* alleles and age (n = 427).

	β	p	95% CI
Intercept	47	2.1E-114	44, 50
***APOE* allele (ε4 carrier as reference)**			
ε3/ε3	0.79	0.21	-0.44, 2.0
ε2 carrier	2.5	0.0091	0.63, 4.4
Age	-0.16	3.2E-9	-0.21, -0.11

B. As in A. with education (n = 412).

	β	p	95% CI
Intercept	43	3.0E-93	40, 46
***APOE* allele (ε4 carrier as reference)**			
ε3/ε3	1.0	0.10	-0.20, 2.2
ε2 carrier	2.1	0.023	0.30, 4.0
Age	-0.14	9.8E-8	-0.19, -0.089
Education level (Elementary as reference)			
Secondary	3.0	1.6E-5	1.7, 4.4
University	5.1	1.7E-11	3.6, 6.5

C. As in B. with diabetes mellitus (n = 412).

	β	p	95% CI
Intercept	43	4.7E-94	40, 46
***APOE* allele (ε4 carrier as reference)**			
ε3/ε3	1.1	0.065	-0.070, 2.3
ε2 carrier	2.3	0.016	0.42, 4.1
Age	-0.13	3.5E-7	-0.18, -0.082
Education level (Elementary as reference)			
Secondary	2.9	3.3E-5	1.5, 4.2
University	4.8	1.8E-10	3.4, 6.3
Diabetes mellitus	-1.9	0.010	-3.4, -0.46

D. As in C. with hyperlipidemia (n = 395).

	β	p	95% CI
Intercept	44	5.1E-89	41, 47
***APOE* allele (ε4 carrier as reference)**			
ε3/ε3	1.2	0.055	-0.026, 2.4
ε2 carrier	2.3	0.019	0.38, 4.1
Age	-0.14	8.3E-7	-0.19, -0.085
Education level (Elementary as reference)			
Secondary	2.7	9.6E-5	1.4, 4.1
University	4.8	7.9E-10	3.3, 6.3
Diabetes mellitus	-2.1	0.0066	-3.5, -0.58
Hyperlipidemia	-0.16	0.81	-1.4, 1.1

APOE, apolipoprotein E gene; BNIS, Barrow Neurological Institute Screen for higher cerebral functions; CI, confidence interval; elementary, completed elementary school or lower; secondary, completed upper secondary school, trade school or college; university, completed university or research education.

coefficient was also significant (β = 1.3, p = 0.037). The betas for hyperlipidemia were not statistically significant in any of the models. Adjusted R^2 values were 0.088 for the model in **Table 5 A**, 0.183 in **B**, 0.194 in **C** and 0.203 in **D**. After adding NIHSS at baseline to the model in **D**, the value was 0.273, and after exclusion of recurrent stroke it was 0.260.

Interactions between *APOE* alleles, age and physical activity

BNIS score was multiply linearly regressed by *APOE* alleles ($\beta_{\varepsilon 2}$ = 7.2, p = 1.2E-04; $\beta_{\varepsilon 3}$ = 1.9, p = 0.11), median-centered age (β = -0.26, p = 9.1E-06) and physical activity level at the 7-year follow-up (β_{high} = 6.2, p = 3.3E-6; $\beta_{moderate}$ = 3.9, p = 0.0012) as well as the three

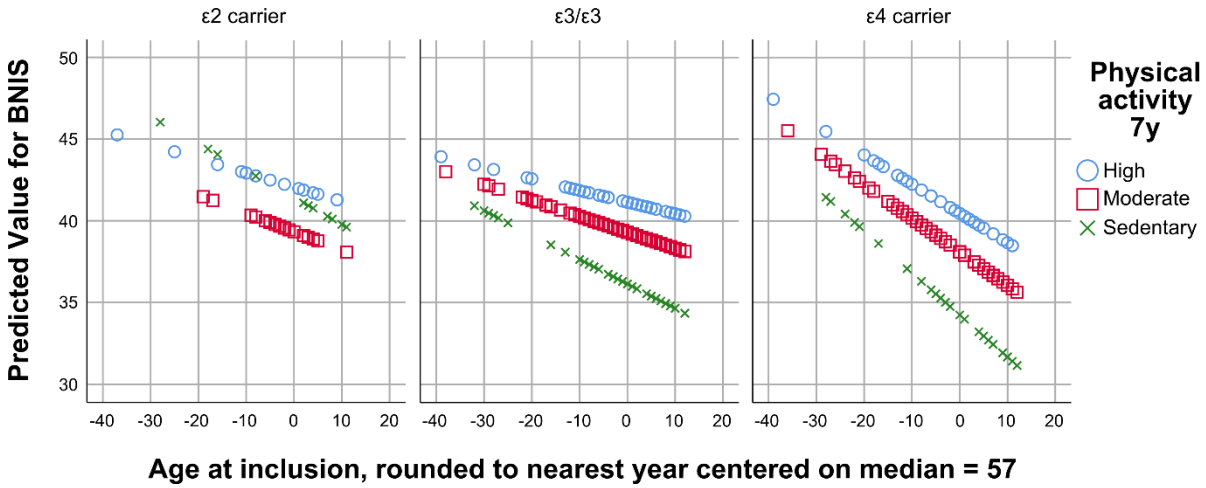

Figure 2. Predicted values for Barrow Neurological Institute Screen for higher cerebral functions (BNIS) score from linear regression by apolipoprotein E gene (*APOE*) alleles, age and physical activity with all three two-way interactions present (n = 419). High: regular, tough or competitive physical activity; moderate: moderate physical activity; sedentary: sedentary physical activity; y, years.

two-way interactions. The reference groups were ε4 carriers and sedentary physical activity. The predicted values are displayed in **Figure 2**, separated by the independent variables to illustrate the interactions. The association between age and BNIS was statistically significantly different in ε3 homozygotes (β = 0.11, p = 0.048). The association between *moderate* as well as *high* physical activity to BNIS was also statistically significantly modified by ε2 carriage ($\beta_{\varepsilon2 \times \text{moderate}}$ = -6.0, p = 0.010; $\beta_{\varepsilon2 \times \text{high}}$ = -5.6, p = 0.029). The adjusted R^2 was 0.167.

APOE alleles and serum levels of NfL

In the controls, one-way ANOVA revealed no difference between the *APOE* allele groups in logarithmized serum NfL levels measured at baseline. The same type of analysis on cases showed no statistically significant differences for serum NfL measured in the acute phase, at three months or at seven years after stroke. **Table 6** displays the means and standard deviations for the *APOE* allele groups as well as ANOVA results.

In a linear regression model for the controls, with age at baseline and *APOE* allele group as independent variables and logarithmized NfL as the dependent variable, the beta values were not significant for ε2 carriers vs. ε4 carriers (β = 0.066, p = 0.12) or for ε3 homozygotes vs. ε4 carriers (β = 5.2E-04, p = 0.99), though it was highly significant for age (β =

Table 6. Base 10-logarithmized serum levels of neurofilament light chain (NfL) for the *APOE* allele groups.

		n	ε2 carrier	ε3/ε3	ε4 carrier	Total	*p*	*F* (*dfb*, *dfw*)
Controls		580	1.19 (0.29)	1.13 (0.35)	1.14 (0.34)	1.14 (0.34)	0.34	1.1 (2, 577)
Cases	**Acute**	474	1.91 (0.53)	1.87 (0.60)	1.90 (0.61)	1.88 (0.59)	0.79	0.2 (2, 471)
	3 months	530	2.04 (0.50)	1.97 (0.53)	1.97 (0.55)	1.98 (0.53)	0.62	0.5 (2, 527)
	7 years	211	1.35 (0.35)	1.25 (0.38)	1.28 (0.37)	1.27 (0.37)	0.48	0.7 (2, 208)

APOE, apolipoprotein E gene; n, number of individuals; *p*, *p* value for difference from analysis of variance (ANOVA) tests; *F*, *F* statistic from ANOVA tests; *dfb*, degrees of freedom between groups; *dfw*, degrees of freedom within groups. All data are presented as mean (standard deviation).

0.016, p = 7.7E-35). Similarly, for cases, the betas for age were significant and the betas for *APOE* were non-significant in regression models for the NfL levels at three months (β_{age} = 0.0085, p = 1.7E-04) and at seven years (β_{age} = 0.022, p = 2.7E-23). When linearly regressing NfL by *APOE* alleles and age in the BNIS study population, there were again no significant betas for the *APOE* allele groups at any timepoint (all $|\beta| < 0.3$, all $p > 0.1$) and age was significant for 7-year levels (β_{7y} = 0.022, p = 2.7E-23; β_{3m} = 0.0060, p = 0.054; β_{acute} = -0.0041, p = 0.33).

Interaction between *APOE* alleles and serum levels of NfL

BNIS score was linearly regressed by mean-centered logarithmized serum NfL levels measured at the 3-month follow-up (β_{NfL} = -6.5, p = 4.2E-08) and *APOE* alleles ($\beta_{\varepsilon 2}$ = 2.3, p = 0.080; $\beta_{\varepsilon 3}$ = 0.34, p = 0.69) as well as by the two-way interaction ($\beta_{\varepsilon 2 \times NfL}$ = 0.21, p = 0.93; $\beta_{\varepsilon 3 \times NfL}$ = 4.4, p = 0.0043). So, there was a statistically significant difference between ε3 homozygotes and ε4 carriers in the relation between serum NfL levels and BNIS. **Figure 3** shows the predicted values from the regression by the independent variables. The adjusted R^2 was 0.139. The interaction term also survived correction for age, education, diabetes mellitus and baseline NIHSS ($\beta_{\varepsilon 3 \times NfL}$ = 3.5, p = 0.011).

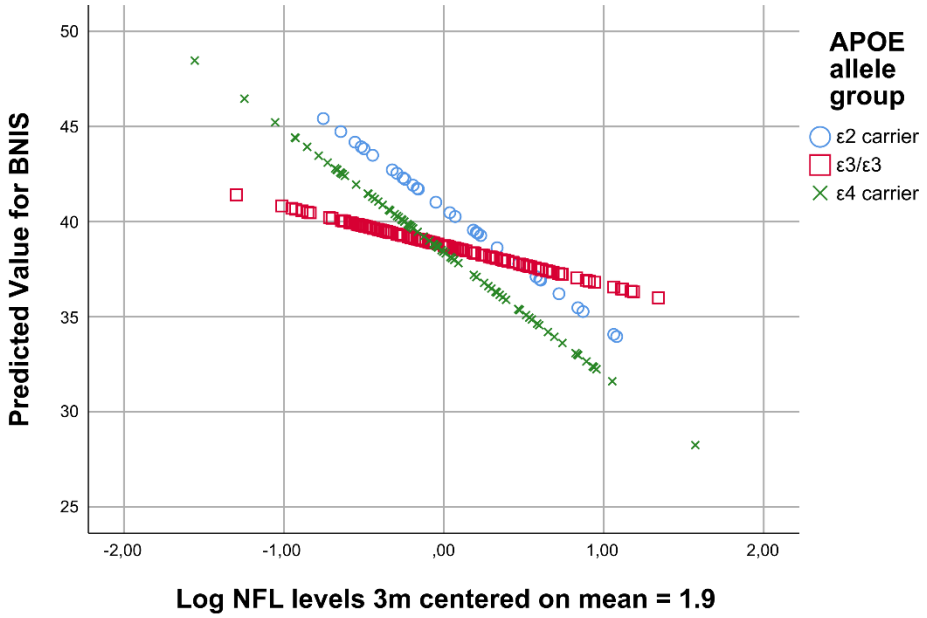

Figure 3. Predicted values for Barrow Neurological Institute Screen for higher cerebral functions (BNIS) score from linear regression by apolipoprotein E gene (*APOE*) alleles and logarithmized 3-month neurofilament light chain (NfL) levels with the two-way interaction present (n = 254). Log, logarithmized; m, months.

Hyperlipidemia and lipids

In order to test mediation, binary logistic regression of hyperlipidemia by *APOE* alleles, and multiple linear regression of BNIS by hyperlipidemia and *APOE* alleles together were carried out. The association between hyperlipidemia and BNIS score did not reach statistical significance when controlling for *APOE* alleles ($\beta = -1.3$, $p = 0.050$), although it did on its own ($\beta = -1.6$, $p = 0.018$). The difference between ε3 homozygotes vs. ε4 carriers was not statistically significant in any of the models, while ε2 carriers vs. ε4 carriers was statistically significant in both models. This is illustrated in **Figure 4** in the form of a directed acyclic graph (DAG) for ε2 carrier vs. ε4 carrier coefficients. **Table 8 A** and **D** (Appendix I, page 47) show the full models, with subtable **B** and **C** being the univariate regressions of BNIS.

Similar mediation analyses were made with *APOE* alleles and acute stage total cholesterol, LDL and HDL, but none of their results supported a mediation hypothesis (data not shown). High acute HDL (but not LDL and cholesterol) was significantly associated to a high BNIS score ($\beta_{HDL} = 2.5$, $p = 0.0029$). And overall, ε4 carriers had statistically significantly higher acute cholesterol and LDL levels than ε2 carriers, but no significant difference was

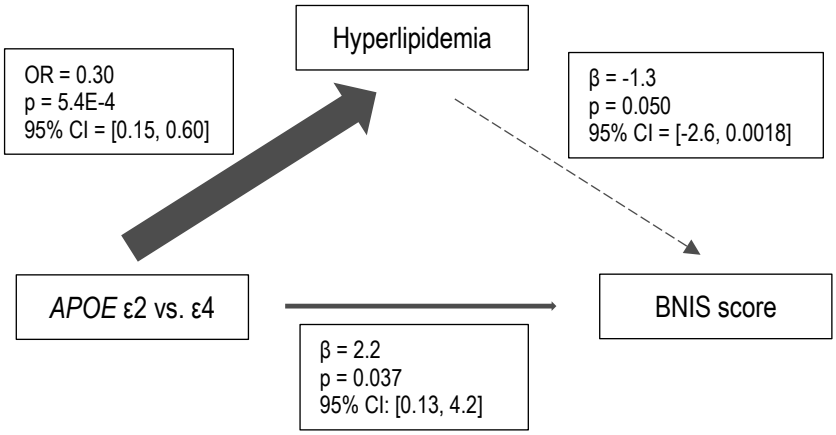

Figure 4. Directed acyclic graph of mediation analysis of hyperlipidemia on the relationship between apolipoprotein E gene (*APOE*) ε2 carriers vs. ε4 carriers and Barrow Neurological Institute Screen for higher cerebral functions (BNIS) score (n = 408). OR, odds ratio; CI, confidence interval.

found for HDL levels. However, in a linear regression of BNIS score by *APOE* alleles ($|\beta_{\varepsilon 2 \text{ and } \varepsilon 3}| < 2.5$, $p > 0.05$) and acute HDL levels centered on the mean of 1.3 ($\beta = 4.4$, $p = 0.0012$), there was a significant interaction between ε3 vs. ε4 and HDL ($\beta = -3.7$, $p = 0.038$). This interaction term remained significant after correcting for age, education, diabetes mellitus and baseline NIHSS ($\beta = -3.2$, $p = 0.047$).

Discussion

Main findings

In this study, we primarily investigated the relationship between *APOE* alleles and long-term cognitive outcome after ischemic stroke as measured by BNIS in young and middle-aged stroke survivors. First and foremost, a statistically significant difference in BNIS score between ε2 carriers and ε4 carriers was demonstrated. This difference survived correction for age, education, diabetes mellitus, hyperlipidemia and stroke severity (NIHSS) at baseline. After excluding those with recurrent stroke, the difference between ε3 homozygotes and ε4 carriers was also statistically significant when correcting for the previously mentioned factors.

In **Table 7**, the characteristics of nine studies which have investigated the relationship between *APOE* alleles and cognitive outcome in a stroke survivor population are summarized. Overall, most were conducted in countries with populations of primarily Western European origin, had less than 200 participants [27, 28, 39-42, 71, 72] (one having 355 [29]), included older patients (mean age between 62 and 81 years [27-29, 39-42, 72], except one with a mean age of 57 years [71]) who had a moderate stroke severity at baseline (average NIHSS between 2 and 7 points [27-29, 39-42, 71, 72]). Four of them included ischemic stroke patients specifically [39, 40, 71, 72].

Compared to these studies, our study was larger (over 400 participants for the main analyses) and included younger patients (median age 57 years). Similar to previous studies, our sample had relatively mild strokes (NIHSS median 3 points) and was conducted in a Western European country (Sweden). The previous study that included patients with a similar age as in the present study (mean age 57 years) had a low number of participants (n = 68) and included a South Korean population [71].

As for the studies that assessed cognitive function at one timepoint, the results of our study are most in line with the studies with follow-up within one year [27, 28, 39]. Two of these studies looked at cognitive score below or above a cutoff value and found an association between ε4 carriage and worse cognitive performance [27, 28]. It should be noted that these two studies were done on the same study population (the latter being a follow-up study) and also did not study ischemic stroke specifically [27, 28]. The third study was on ischemic stroke and reported a lower verbal memory score after three months in the ε4 carrier group [39].

However, the latter study also found a higher executive function score in the ε4 carrier group at 12 months as well as an improvement in ε4 carriers at 12 months compared to their score at 3 months [39]. One study with a two-week follow-up reported significantly higher ε4

Table 7. Overview of studies on the association between *APOE* and cognition in stroke survivor populations.

	Number (case + control)	Age, average (range)	Country	Type of stroke	Inclusion criteria	NIHSS, average	*APOE* groups	Cognition results
Allan et al. [29]	355 (no controls)	M: 80 SD: 4 (≥ 75)	UK	All	In stroke register; MMSE ≥ 24 at 3 mo.; no dementia, severe aphasia or other disability	N/A	ε4 carrier* vs. others	No sign. diff. incident dementia
Ballard et al. [41]	159 (137+22)	M: 81 SD: 4 (≥ 75)	UK	All	In stroke register; no dementia, severe aphasia or other disability	N/A	ε4 carrier* vs. others	Sign. decline in CIND group
Bour et al. [42]	92 (no controls)	M: 68 SD: 13 (≥ 40)	Netherlands	All (first-ever supratentorial)	< 48 h since stroke; MMSE > 15 at baseline; fluent in Dutch; no dementia, severe aphasia or other disability	N/A	ε4 carrier* vs. others	No sign. diff. decline
Lee et al. [71]	68 (no controls)	M: 57 SD: 9-11	South Korea	Ischemic	Male sex; no intake of drugs that affect homocysteine; low coffee/alcohol consumption; no cigarette smoking	N/A	ε4 carrier* vs. others	No sign. diff. in score or decline
Qian et al. [40]	192 (152+40)	M: 62-75 (40, 88)	China	Ischemic (first-ever)	CT/MRI verified; no dementia, cognitive impairment or severe aphasia	M: 3-5	ε4 carrier* vs. others + alleles	No sign. diff. adjusted but unadjusted
Tene et al. [72]	182 (no controls)	M: 67 SD: 10 (> 50)	Israel	Ischemic (first-ever)	NIHSS < 17; no cognitive impairment before stroke, severe aphasia, other disability, drug abuse, psychiatric disorder, steroidal medication within 6 mo.	Md: 2	ε4 carrier* vs. others	No sign. diff. score, but sign. interaction with cortisol
Wagle et al. (2009) [27]	152 (no controls)	M: 77 SD: 11	Norway	All	Fluent in Norwegian; no severe disability, drug abuse or psychiatric disorder	M: 7	ε4 carrier* vs. others	Sign. score overall, memory, language
Wagle et al. (2010) [28]	104 (no controls)	M: 76 SD: 11	Norway	All	Fluent in Norwegian; no severe disability, drug abuse or psychiatric disorder	Md: 2-7	ε4 carrier* vs. others	Sign. score in verbal memory and overall
Werden et al. [39]	40 (20+20)	Md: 67 (52, 84)	Australia	Ischemic	CT/MRI verified; able to do MRI; < 3 months since stroke; no dementia, neurodegenerative disorder or severe aphasia	Md: 2-3	ε4 carrier* vs. others	Sign. score in verbal memory, but better executive

* Including the ε4/ε2 genotype; *APOE*, apolipoprotein E gene; NIHSS, National Institutes of Health Stroke Scale; M, mean; Md, median; SD, standard deviation; mo., months; sign., significant (more impaired unless stated otherwise); diff. difference; VM, verbal memory; CI, cognitive impairment; CIND, cognitive impairment no dementia; CT, computer tomography, MRI, magnetic resonance imaging; MMSE, Mini Mental State Examination; UK, United Kingdom.

allele frequencies in groups with cognitive impairment when the ε2, ε3 and ε4 alleles were separated, but there was no significant difference in cognitive outcome between ε4 carriers vs. non-carriers when adjusting for age and education [40].

Our results stand in contrast to the study with the most similar follow-up time (8 years) [29]. The study, which had dementia incidence as its outcome variable, did not find ε4 to be a risk factor [29]. The difference in results could be attributed to the difference in study design, since that study included stroke survivors of higher ages and recorded the incident cases of dementia. There may be differences in how *APOE* alleles affect the passage over the threshold for what is classified as dementia vs. how they affect cognitive performance as measured by BNIS, which is a continuous variable.

There have also been studies on cognitive decline over time in ε4 carriers and non-carriers among stroke survivors. These studies had a follow-up of 1-2 years and came to different conclusions. One is in line with our results, reporting a significantly greater decline in ε4 carriers, however only in the *cognitive impairment no dementia* (CIND) group [41]. We did not categorize our participants according to cognition in this manner, which of course makes direct comparisons difficult. Three other studies show dissimilar results to ours, with no decline or difference in decline observed [42, 71, 72]. It is however entirely possible that *APOE* does not affect the speed of cognitive decline, while still having an effect on the overall cognitive function. Detection of a difference in scores between two time points may also be methodologically more challenging and subject to more variation than determining cognitive function at one timepoint.

Our follow-up time was indeed longer than in most of the studies mentioned. The time points of testing and time to follow-up are obviously important since one would generally expect cognitive function to improve in the short-term e.g. months after stroke, but decline with

age in the long-term span of years. Investigating change in cognitive scores over time among younger stroke patients presents an opportunity for future research.

Our main independent variable, *APOE* alleles, was different from previous studies, which all looked at ε4 carriage vs. non-carriage, while we compared ε4 carriers to ε3 homozygotes and ε2 carriers separately and excluded the ε4/ε2 genotype. Having a lower resolution in the predictor will result in larger groups, but may also obscure a possible real association between the *APOE* alleles and cognition. Examining the ε2 allele separately from ε3 homozygotes remains a subject of interest, since our results indicate that ε2 carriers separate themselves the most from the other allele groups in cognitive performance post-stroke.

BNIS as a test can of course not differentiate between different etiologies of cognitive impairment. It may identify consequences of ischemic stroke as well as an ongoing development of Alzheimer's disease, for example. However, the impairment is detrimental to the stroke survivor regardless of cause and thus equally important to detect. Dementia or Alzheimer's disease among BNIS participants was not considered as a separate variable or explicitly asked about, although cases with severe dementia were excluded at follow-up (see page 17). It is possible that some participants with mild or early-stage dementia may have gone undetected and successfully performed BNIS, which in turn may have affected our results. However, due to the nature of the pre-screen test, we consider it unlikely that anyone with moderate dementia or worse would have passed it and thus proceeded to perform BNIS.

Another point to contemplate is whether these statistically significant differences in BNIS score are also clinically significant. A recommended cut-off for classifying an individual as cognitively impaired is less than 47 points on BNIS [73]. As such, mean differences of 1-3 points between allele groups, such as those we found, are meaningful in that regard as they could place an individual in another category of cognitive function. Furthermore, if the effect of age is assumed to be 0.14 points less each year (as in **Table 5 D**), the difference

between allele groups could be said to correspond to 10-15 years of aging, which we think most could agree is a consequential difference. Finally, one could adopt the perspective that these differences are indicative of a higher *risk* of developing further cognitive impairment, and perhaps even dementia, a risk which of course is clinically relevant.

Sex

When stratifying by sex, our study revealed a statistically significant difference in BNIS score between the *APOE* allele groups in women, but not in men. However, there was no statistically significantly different association between *APOE* alleles and BNIS score dependent on sex, with or without adjusting for age. From our literature search, we have not been able to find similar analyses on a pure stroke survivor population. However, results of one population-based study show an association between ε4 carriage and cognitive decline in women between the ages 70 and 80 years, but not in men [74]. Somewhat contrary to our results, the relationship between stroke and memory test score decline has been described as stronger in men and those *without* the ε4 allele in the general population [52]. There is also one study on individuals without a history of stroke which reported that male ε4 carriers had greater declines in verbal memory than non-carriers, and that female ε4 carriers had worse executive function, and they also found an interaction between male sex and short-time recall [75].

Physical activity

In the interaction analysis for physical activity, moderate to high physical activity was associated to statistically significantly higher BNIS score in ε4 carriers compared to ε2 carriers when adjusting for age. In ε4 carriers the association between age and BNIS score was significantly stronger than in ε3 homozygotes when physical activity was considered. These results indicate that the association of physical activity to cognitive performance differs based on the presence of *APOE* alleles. This difference is reflected in the divergent slopes for the

predicted values between the *APOE* allele groups in **Figure 2**. Broadly speaking, our results suggest that physical activity matters more in ε4 carriers (i.e. the group with high genetic risk) than in ε2 carriers. However, it is of note that the ε2 carrier group was small (see Methodological considerations below).

We have not found studies on stroke survivors whose results agree with this result. Contrasting with our results, one study on stroke survivors reported that carriage of the ε4 allele did not modify the link between physical exercise and cognition [71] On the other hand, this was a small study with a short-term follow-up (see **Table 7**) that had several outcome variables, and may not have been suited to detect any possible association between *APOE* alleles and cognition. Our results generally agree with those of three large-scale general population studies that looked at genetic and modifiable risk factors for cognitive impairment. According to these, both high genetic risk (including ε4 carriage) and unfavorable lifestyle separately confer a higher risk of dementia or lower cognitive scores [76-78]. Also in line with our results, one of these studies found that those with high genetic risk were affected the most by lifestyle [77]. However, the results of one study point in the opposite direction, concluding that unfavorable lifestyle seems to matter only in those with low genetic risk [76].

Neurofilament light chain

There was no statistically significant difference in serum levels of NfL between the *APOE* allele groups at any time point either in cases or in controls when correcting for age, which was generally associated to NfL levels. However, when analyzing interactions, we found that cases who were ε4 carriers displayed a significantly stronger correlation between three-month NfL and BNIS compared to ε3 homozygotes. Others have studied the relationship between *APOE*, NfL and cognition. One study revealed a similar association between NfL levels and cognitive decline in Alzheimer's disease patients, and NfL levels were higher for ε4 carriers in the mild cognitive impairment and Alzheimer's disease groups, but not in

controls [79]. Another study observed a similar association between high NfL levels and poor cognitive performance in Alzheimer and Parkinson patients, but NfL levels were similar between ε4 carriers and non-carriers [80]. Based on our literature research, the relationship between *APOE* alleles and serum NfL levels has not previously been studied in a stroke survivor population, and neither has interactions between the two when it comes to cognition. Thus, this study presents novel information on the relation between NfL and cognition in stroke survivors, while falling in line with the hypothesis that serum NfL levels do not differ between *APOE* allele groups.

NfL has been connected to outcomes besides cognition in stroke survivors. In another study from our group based on SAHLSIS, serum levels of NfL three months after index stroke were shown to predict the extent of neurological impairment and functional outcome after seven years [62]. The results of the same study and another recent study [81] indicate that the peak levels for NfL lie somewhere in the first few weeks after stroke, which may explain why 3-month levels were similarly predictive of the cognitive outcome in this study.

Interestingly, our interaction analysis suggests that the processes which increase NfL in serum, such as neurodegeneration, have a more pronounced effect on cognition in ε4 carriers than in ε3 homozygotes. Some of the processes in the brain which increase serum levels of NfL, for example amyloidopathy, have a greater impact on cognition than others. One possible explanation for the interaction result might thus be a greater relative contribution of amyloid β-caused neuronal destruction to serum levels of NfL in ε4 carriers compared to ε3 homozygotes. The reason for this would be that having the ε4 allele leads to a greater accumulation of amyloid β [36]. On another note, it has been demonstrated that serum NfL levels are associated with cerebral small vessel disease [82], a common contributing factor to cognitive impairment. Thus, although highly speculative, our results could be interpreted as small vessel disease having a larger impact on cognitive function in ε4 carriers. Although we have data on

etiological subtypes according to the TOAST classification, we believe that we are underpowered to investigate this hypothesis in the present study. Hence, further studies are needed to elucidate this matter.

Hyperlipidemia and lipids

As expected, an association between *APOE* alleles and hyperlipidemia was found in our study, an association which is well-established [35]. However, the association between hyperlipidemia and cognition did not survive correction for *APOE* alleles, which means that the data do not support a mediation hypothesis in that case. The same was true for mediation by total cholesterol, LDL and HDL in the acute stage. We did however find that HDL was associated with higher scores on the BNIS. Interestingly, the association between HDL and BNIS was statistically significantly greater in ε4 carriers compared to ε3 homozygotes. This is rather contrary to the result of another study, which showed a positive relationship between HDL and cognitive score in non-ε4 carriers, but not in ε4 carriers, in the general population [83]. However, this was a stratified analysis done on patients above 65 years of age, which may explain the different results.

The E4 isoform of apolipoprotein E, which is encoded by the ε4 allele, binds preferentially to very low-density lipoprotein (VLDL) over HDL [84]. HDL is often called the "good cholesterol" because of its higher ability to remove lipids from atherosclerotic plaques, while LDL is called the "bad cholesterol" because it increases atherosclerosis [84]. In the brain, apolipoprotein E in HDL-like particles has a role in cholesterol transport, which may be important for synaptic plasticity after brain injury [85]. Thus, it could be very tentatively speculated that the presence of the E4 isoform enhances the abilities of HDL and HDL-like particles to exercise their protective effect on the brain and cognitive function.

Effects of *APOE* within the central nervous system

The secondary aim of this study was to search for factors that might contribute to the understanding of how *APOE* alleles influence cognitive performance post-stroke. There is insufficient knowledge about these mechanisms, and what mediates them. As discussed above, we studied circulating biomarkers of neuronal damage, NfL and lipid levels, by virtue of their ease of measurement. But naturally, the processes by which *APOE* affects cognition almost certainly occur locally within the central nervous system (CNS).

Several other biomarkers that hold promise for this purpose exist. For example, the biomarkers tau and GFAp measured in the acute stage of acute ischemic stroke have recently been shown be associated to the functional outcome after three months [86]. These biomarkers reflect the degeneration of different cell types within the CNS: NfL that of long myelinated axons in the white matter, tau that of non-myelinated axons in the grey matter and GFAp that of astrocytes, a type of glial cells [87]. It is natural to assume that the degeneration of these distinct cell types has different effects on cognitive function after stroke. It would therefore be of interest in the future to also study tau and GFAp in relation to *APOE* alleles and post-stroke cognition. A challenge for the future is to find additional blood biomarkers that reflect processes within the CNS.

Methodological considerations

A methodological strength of this study was that directly genotyped *APOE* data was available for the vast majority of the individuals who were part of the main BNIS analyses. However, given that we found a very high and statistically significant level of agreement between imputation and direct genotyping, we conclude that imputed data infer APOE alleles with high accuracy.

Having BNIS, a continuous score for cognitive function with a demonstrated stroke population validity, as the outcome strengthens the study. A dichotomous outcome such as

dementia or mild cognitive impairment incidence is both less sensitive and more extensively studied as a cognitive outcome for *APOE* alleles. With BNIS, even small changes in cognition can be detected.

The relatively low missing percentages for the baseline and 7-year characteristics make it easier to draw conclusions about the attributes of the participants and assess possible bias. With regards to baseline characteristics, the only significant difference between the *APOE* allele groups was in hyperlipidemia prevalence, making possible confounding effects for other variables less likely. It is also reassuring that the *APOE* genotype frequencies were in line in with Hardy-Weinberg equilibrium, i.e. they did not differ systematically from what could be expected in a randomly mating population.

With respect to attrition rates, 12% of included stroke participants died during follow-up, and 22% did not participate in the BNIS testing for other reasons. This loss to follow-up introduces a risk of bias to the results. In fact, BNIS participants were significantly less likely to smoke or have diabetes mellitus and they had significantly less severe strokes (lower NIHSS at baseline) compared to BNIS non-participants. In broader terms, the BNIS participants seem to have been healthier at stroke onset. This prevents the generalization of the results of this study to all stroke survivors.

The fact that the difference between ε2 carriers and ε3 homozygotes was not evaluated in the linear regressions is another limitation of the study. However, given three groups with one reference group, one comparison will always be left out. An alternative would have been to analyze the groups pairwise, but this would have reduced the sample size. Excluding those with the ε4/ε2 genotype means that the results cannot be generalized to that group, albeit a small one. The fact that the ε2 carrier group was small is also a limitation to the interpretation of the results for this group, and larger studies are clearly needed to evaluate our findings.

Data on cognitive function as measured by BNIS at baseline was not collected in SAHLSIS. This makes us unable both to look at change in cognitive function over time, but also to adjust for individual variation in cognitive function. Lastly, we did not have a group of healthy controls that participated in the seven-year follow-up. Having a such a control group who had performed BNIS would have made it possible to explore differential effects of *APOE* alleles on cognition depending on whether a person has had a stroke or not.

Conclusions and Implications

The results of this study indicate that the *APOE* alleles carried by a stroke survivor are associated with their cognitive performance many years after they suffered a stroke. Compared to stronger predictors such as age and education level, they explain only a small fraction of the variation in cognition. However, our results also suggest that *APOE* alleles alter the association between other factors of importance for cognition and cognitive performance. Recently, several additional genetic variants that associate to cognitive function have been discovered [88]. Consequently, *APOE* alleles and possible additional genetic variants, together with clinical variables, hold promise as predictors of cognitive impairment in clinical practice. This could in the future lead to a more effective allocation of resources in post-stroke preventative measures, as well as more personalized rehabilitation, for a common but often neglected issue among stroke survivors. As mentioned on page 7, strategies for addressing post-stroke cognitive impairment are the subject of ongoing research.

With regards to the mechanisms for how *APOE* alleles affect post-stroke cognition, our results indicate that future research on blood biomarkers of neuronal damage, such as NfL, are of interest. How the protective effect of HDL on cognition could vary depending on *APOE* alleles also merits further study. Other exciting prospects for explaining these mechanisms not explored in this work are amyloid β levels, which of course tie into the relationship between post-stroke cognitive decline and Alzheimer's disease. The importance of examining

the three *APOE* alleles separately in coming studies is also affirmed by this study. It is apparent that ε2 carriers and ε3 homozygotes are not a homogenous group when it comes to their relationship with cognitive impairment.

Populärvetenskaplig sammanfattning (Summary in Swedish)

APOE-alleler i relation till kognitivt utfall efter ischemisk stroke

Stroke är en av de främsta orsakerna till att människor dör i förtid och till att de får funktionsnedsättningar. Oroväckande nog verkar antalet nya fall bland yngre personer öka. Ischemisk stroke (hjärninfarkt) är den vanligaste formen av stroke och orsakas av att ett blodkärl i hjärnan blockeras, vilket leder till syrebrist i hjärnan och vävnadsskada. Kognitiva funktionsnedsättningar, till exempel försämrad språkförmåga, uppmärksamhet och minne, är vanliga efter en stroke, och kunskapen om vilka faktorer som påverkar dessa är begränsad.

Apolipoprotein E (*APOE*) är en gen (arvsanlag) med tre alleler (varianter), ε2, ε3 och ε4 (ε: epsilon). ε4-allelen är kopplad till ökad risk för Alzheimers sjukdom, den vanligaste orsaken till demens, medan ε2-allelen är kopplad till lägre risk. Resultat från tidigare studier pekar mot att *APOE*-alleler även kan ha samband med hur kognitiva funktioner utvecklas efter stroke. Andra faktorer har också betydelse, bland annat ålder, diabetes, fysisk aktivitet och utbildningsnivå.

Denna studies huvudsakliga syfte var att undersöka om det fanns någon skillnad i kognitiv funktion bland yngre och medelålders strokepatienter flera år efter insjuknandet i ischemisk stroke beroende på vilken *APOE*-allel de bar på. Det underordnade syftet var att utforska faktorer som kan hjälpa oss att förstå hur *APOE*-genen påverkar kognition. Studien baseras på Sahlgrenska akademins Studie av Ischemisk Stroke (SAHLSIS). Inom ramen för denna studie har patienter under 70 år med akut ischemisk stroke inkluderats och följts upp efter sju år. Information inhämtades från besök hos läkare och sjuksköterska, enkäter, journaler och analys av blodprover. Vid uppföljningen efter sju år deltog drygt 400 studiedeltagare i ett kognitivt

test, BNIS, som testar flera kognitiva funktioner som ofta är påverkade efter stroke. Informationen analyserades statistiskt för att se vilka samband och skillnader som var betydelsefulla.

Vi fann en skillnad i kognitiv funktion mellan de med ε4-allelen (ε4-bärare) jämfört med de med ε2-allelen (ε2-bärare). Denna skillnad fanns kvar även när vi tog hänsyn till studiedeltagarnas ålder, utbildningsnivå, om de hade diabetes eller höga blodfetter samt strokens svårighetsgrad. När vi uteslöt de som fått en ny stroke innan uppföljningen så kunde vi även se en skillnad mellan ε4-bärare och de med två ε3-alleler. Vilken allel studiedeltagarna hade gjorde även skillnad för andra faktorers koppling till den kognitiva funktionen. Till exempel var måttlig till hög fysisk aktivitet jämfört med låg aktivitet kopplat till bättre kognitiv funktion hos ε4-bärare jämfört med ε2-bärare.

Våra resultat kan vara till hjälp för kommande forskare när de utformar sina studier av kognitiv funktion efter stroke. I framtiden hoppas vi att man kan använda sig av resultat liknande dessa för att förbättra den kognitiva återhämtningen hos människor som drabbas av stroke, till exempel för att identifiera personer som har en högre risk att återhämta sig sämre. På lång sikt kan en ökad förståelse av *APOE*-genens koppling till kognition hos strokepatienter leda till att man utvecklar nya läkemedel som bromsar kognitiv nedsättning och på så sätt ökar patienters självständighet och livskvalitet.

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Appendices

Appendix I: Hyperlipidemia mediation analysis

Table 8. Mediation analysis of hyperlipidemia on the association between *APOE* alleles and BNIS (n = 408).

A. Hyperlipidemia by *APOE* alleles.

	OR	p	95% CI
***APOE* allele (ε4 carrier as reference)**			
ε3/ε3	0.96	0.88	0.60, 1.6
ε2 carrier	0.30	5.4E-4	0.15, 0.60

B. BNIS by hyperlipidemia.

	β	p	95% CI
Intercept	40	7.4E-236	39, 41
Hyperlipidemia	-1.6	0.018	-2.9, -0.27

C. BNIS by *APOE* alleles.

	β	p	95% CI
Intercept	39	8.3E-237	37, 40
***APOE* allele (ε4 carrier as reference)**			
ε3/ε3	0.64	0.34	-0.68, 2.0
ε2 carrier	2.5	0.013	0.53, 4.6

D. BNIS by *APOE* alleles and hyperlipidemia.

	β	p	95% CI
Intercept	39	2.6E-190	38, 41
***APOE* allele (ε4 carrier as reference)**			
ε3/ε3	0.63	0.35	-0.68, 1.9
ε2 carrier	2.2	0.037	0.13, 4.2
Hyperlipidemia	-1.3	0.050	-2.6, 0.0018

APOE, apolipoprotein E; BNIS, Barrow Neurological Institute Screen for higher cerebral functions; OR, odds ratio; CI, confidence interval. The *p* values have not been corrected for multiple testing.

Appendix II: Relevant excerpts from the seven-year follow-up questionnaires

Vilken högsta utbildning har du?

- ☐ Ej fullgjort grundskola eller folkskola
- ☐ Grundskola eller folkskola
- ☐ Gymnasium, fackskola eller folkhögskola
- ☐ Högskola eller universitet
- ☐ Forskarutbildning

Totalt antal år som du gått i skolan: _____

Är du för närvarande: *(Du kan kryssa för flera alternativ)*

- ☐ Yrkesarbetande heltid
- ☐ Yrkesarbetande deltid
- ☐ Studerande heltid
- ☐ Studerande deltid
- ☐ Sjukskriven heltid
- ☐ Sjukskriven deltid
- ☐ Sjukersättning heltid
- ☐ Sjukersättning deltid
- ☐ Ålderspensionär
- ☐ Arbetssökande
- ☐ Avtalspensionär
- ☐ Annat *(specificera)*: _____

Har ditt yrkesarbete (din arbetssituation) förändrats efter du fick stroke?

- ☐ Nej
- ☐ Ja , på grund av min stroke
- ☐ Ja, på grund av min ålder
- ☐ Ja, på grund av *(specificera)* _____

Känner du oro och ångest?

- ☐ Nästan aldrig
- ☐ Ibland
- ☐ Ofta
- ☐ Ständigt

Röker du?

- ☐ Nej, jag har aldrig rökt
- ☐ Ja
- ☐ Nej, jag slutade för _____ år sedan

Har du efter din stroke haft långvarig smärta i den del av kroppen som du hade symtom från vid strokeinsjuknandet?

- ☐ Nej (*Om nej, gå vidare till bokstaven D nedan*)
- ☐ Ja

Har du fortfarande smärtor?

- ☐ Nej
- ☐ Ja

Om ja, kan du beskriva smärtan och ange var smärtan finns?

_____

_____

_____

Tar du någon medicin på grund av dessa smärtor?

- ☐ Nej *(hoppa till bokstaven C nedan)*
- ☐ Ja

Om ja, vilken medicin då?

_____

F. Har du drabbats av ny stroke (propp/infarkt eller blödning), under de senaste sju åren?

☐ Nej *(hoppa till bokstaven G nedan)*

☐ Ja

Om ja, vilken typ av stroke var det? *Om du är osäker, ange ungefärligt år. Du kan kryssa i flera alternativ.*

☐ Propp/infarkt år _____

☐ Blödning år _____

☐ Annat år _____ Precisera _____

☐ Vet ej

Om ja, vilket eller vilka sjukhus var du intagen på? _____

_____

G. Har du efter din stroke haft övergående neurologiska symtom från någon del av kroppen (*exempelvis domningar, stickningar, förlamningssymtom, ryckningar, störning av talet eller synen*)?

☐ Nej (*hoppa till bokstaven H nedan)*

☐ Ja

Har du sökt läkare, vårdcentral eller sjukhus för dessa övergående symtom?

☐ Nej

☐ Ja

Om ja, vilken läkare, sjukhus eller vårdcentral? _____

_____

_____

Kan du beskriva symtomen? _____

_____

_____

Vilken diagnos fick du? _____

_____

_____

H. Efter din stroke, har någon av dina syskon eller föräldrar haft:

stroke?

☐ Nej

☐ Ja, blodpropp/infarkt

☐ Ja, blödning

☐ Vet ej

TIA (övergående strokeliknande anfall)?

☐ Nej

☐ Ja

☐ Vet ej

hjärtinfarkt?

☐ Nej

☐ Ja

☐ Vet ej

Har du av läkare fått diagnosen kärlkramp?

☐ Nej

☐ Ja

Har du haft hjärtinfarkt under de senaste sju åren?

☐ Nej

☐ Ja

Vilket/vilka år? _____

Vilket/vilka sjukhus uppsökte du? _____

Har du av läkare fått diagnosen sömnapnésyndrom?

☐ Nej

☐ Ja, men jag har inte fått någon behandling

☐ Ja, och jag har blivit behandlad med *(ange)*: _____

Har du eller har du haft någon cancersjukdom?

☐ Nej (*hoppa till bokstaven J nedan)*

☐ Ja

Om ja, vilken/vilka? _____

J. Har du eller har du haft någon annan allvarlig sjukdom?

☐ Nej (*hoppa till bokstaven K nedan)*

☐ Ja

Om ja, vilken/vilka?_____

K. Ange namn på de mediciner du tar regelbundet för närvarande

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

☐ Jag tar inga mediciner

HAD

Detta formulär innehåller frågor om hur du har känt dig under den senaste veckan. Besvara frågorna genom att markera det svarsalternativ du tycker stämmer bäst. Om du är osäker, markera det alternativ som känns riktigast.

1. Jag känner mig spänd eller ”uppskruvad”

- ☐ För det mesta
- ☐ Ofta
- ☐ Då och då
- ☐ Inte alls

2. Jag uppskattar samma saker som förut

- ☐ Precis lika mycket
- ☐ Inte lika mycket
- ☐ Bara lite
- ☐ Knappast alls

3. Jag får en slags känsla av rädsla som om någonting förfärligt håller på att hända

- ☐ Alldeles bestämt och rätt illa
- ☐ Ja men inte så illa
- ☐ Lite, men det oroar mig inte
- ☐ Inte alls

4. Jag kan skratta och se saker från den humoristiska sidan

- ☐ Lika mycket som jag alltid kunnat
- ☐ Inte riktigt lika mycket nuförtiden
- ☐ Absolut inte så mycket nuförtiden
- ☐ Inte alls

5. Oroande tankar kommer för mig

- ☐ Mycket ofta
- ☐ Ofta
- ☐ Då och då men inte så ofta
- ☐ Bara någon enstaka gång

6. Jag känner mig glad

- ☐ Inte alls
- ☐ Inte ofta
- ☐ Ibland
- ☐ För det mesta

7. Jag kan sitta i lugn och ro och känna mig avspänd

- ☐ Absolut
- ☐ Oftast
- ☐ Inte ofta
- ☐ Inte alls

8. Jag känner mig som om jag gick på ”lågt varv”

- ☐ Nästan jämnt
- ☐ Mycket ofta
- ☐ Ibland
- ☐ Inte alls

9. Jag får en slags känsla av rädsla som om jag hade ”fjärilar i magen”

- ☐ Inte alls
- ☐ Någon gång
- ☐ Rätt ofta
- ☐ Mycket ofta

10. Jag har tappat intresset för mitt utseende

- ☐ Absolut
- ☐ Jag bryr mig inte så mycket om det som jag borde
- ☐ Jag kanske inte bryr mig om det riktigt så mycket
- ☐ Jag bryr mig precis lika mycket om det som förut

11. Jag känner mig rastlös som om jag måste vara på språng

- ☐ Verkligen mycket
- ☐ En hel del
- ☐ Inte så mycket
- ☐ Inte alls

12. Jag ser fram emot saker och ting med glädje

- ☐ Lika mycket som förut
- ☐ Något mindre än jag brukade
- ☐ Klart mindre än jag brukade
- ☐ Nästan inte alls

13. Jag får plötsliga panikkänslor

- ☐ Verkligen ofta
- ☐ Rätt ofta
- ☐ Inte så ofta
- ☐ Inte alls

14. Jag kan njuta av en bra bok, ett bra radio- eller TV-program

- ☐ Ofta
- ☐ Ibland
- ☐ Inte så ofta
- ☐ Mycket sällan

Hur mycket rör Du dig och anstränger dig kroppsligt på fritiden?

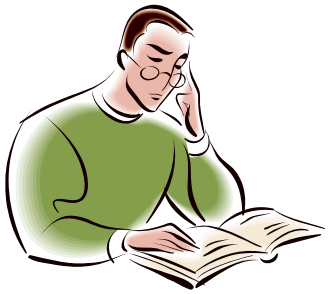
Om Din aktivitet varierar mycket mellan t.ex. sommar och vinter så försök ta ett genomsnitt. Frågan gäller det senaste året.

Kryssa endast i en ruta

Grupp 1 ☐

Stillasittande fritid

Du ägnar dig mestadels åt att läsa, hålla på med dator, se på TV, gå på bio eller annan stillasittande sysselsättning på fritiden.


Grupp 2 ☐

Måttlig motion

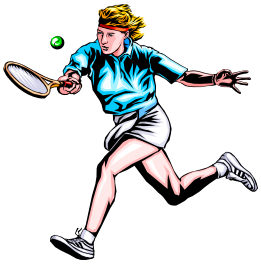
Du promenerar, cyklar eller rör dig på annat sätt under minst fyra timmar i veckan. I detta räknas också gång eller cykling till och från arbetet samt lättare trädgårdsarbete, fiske, bordtennis och bowling.



Grupp 3 ☐

Regelbunden motion och träning

Du ägnar dig åt löpning, simning, tennis, badminton, motionsgymnastik eller liknande. Tyngre trädgårdsarbete och liknande räknas till denna grupp. OBS! Det skall vara i genomsnitt 2-3 tim/vecka.


Grupp 4 ☐

Hård träning eller tävlingsidrott

Du ägnar dig åt hård träning och tävling i löpning, orientering, skidåkning, simning, fotboll, handboll m.m. Detta skall vara regelbundet och flera gånger i veckan.



BNIS

BNI-screening av högre cerebrala funktioner

Barrow Neurological Institute Screen for Higher Cerebral Function (BNIS) George P Prigatano, Ph. D.

Dominant hand		☐ **Vänster**	☐ **Höger**	☐ **Lika**

Förtest

Medvetandenivå/alerthet		**3**	**2**	**1**
Basal språkfunktion		**3**	**2**	**1**
Grad av medverkan		**3**	**2**	**1**

Screeninguppgifter

Flytande tal	Ja=1, nej=0				**1**	**0**	A
Parafasi (ordförväxlingar)	Nej=1, ja=0				**1**	**0**	A
Dysartri (talsvårigheter)	Nej=1, ja=0				**1**	**0**	A
Förståelse	Rör vid den lilla röda cirkeln och den stora vita fyrkanten. Korrekt vid första försöket=2, vid andra=1, annars 0			**2**	**1**	**0**	A
Benämning	Vad är detta? (säng). Ser du att dessa saker är olika, vad heter de? (kudde/lakan eller filt). Vad är detta? (triangel). Vad heter detta (gaffel) och detta (penna). Alla rätt=1 annars 0.				**1**	**0**	A
Visuell objektigenkänning	Peka på den som du använder för att skriva med.				**1**	**0**	D
Repetition	”Nyasfalterad förortsgata” eller ”Nedbrunnen två-familjsvilla”. Korrekt vid första försöket=2, andra=1.			**2**	**1**	**0**	A
Läsning	Tre kronor vann hemmamatchen. Korrekt vid första försöket=1, andra=0,5			**1**	**½**	**0**	A
Skrivning – kopiering	Kopiera meningen ovan				**1**	**0**	A
Skrivning – diktamen	”Tänker du komma hit?”				**1**	**0**	A
Stavning – avvikande	Skridsko				**1**	**0**	A
Stavning – fonetisk	Ledare				**1**	**0**	A
Medvetenhet om minnesstörning	Hus, träd, flyger – antal FP kommer att komma ihåg ___						
Höger-vänsterorientering	(säges – läses ej)				**1**	**0**	B
Rumsorientering – plats	stad, byggnad				**1**	**0**	B
Tidsorientering – datum	dag-mån-år-veckodag				**1**	**0**	B
Konstruktionspraxi– dominant hand	rita ett grekiskt kors				**1**	**0**	D
Dito icke-dominant hand	rita ett grekiskt kors				**1**	**0**	D
Aritmetik - alexi	4 3 = 12				**1**	**0**	A
Aritmetik– dyskalkuli	10+832+963=1805				**1**	**0**	A

Aritmetik – huvudräkning	Säg högt ”fyrtiotvå minus tjugoåtta”. (= ”fjorton”)				**1**	**0**	C
Siffror	– framlänges				**1**	**0**	C
Siffror	– baklänges				**1**	**0**	C
Visuell avsökning, 60 sek	Hur många tvåor? (12) Korrekt vid första försöket=2, andra=1, annars 0.			**2**	**1**	**0**	D
Visuell sekvensering, 3 min	Vilka rader har exakt samma siffror? (rad 3 och 4)				**1**	**0**	D
Mönsterkopiering	Kopiera exakt vad du ser på detta kort				**1**	**0**	D
Mönsteranalys, 30 sekunder	Följ mönstret – vad skall skrivas på dessa två tomma platser? (Svar: ○)				**1**	**0**	D
Inlärning och minne – siffer-symboltest (max 4 min) Använd tid:_____	⊤ = _____ (7) × = _____ (1) + = _____ (5) ⊥ = _____ (4)	**4**	**3**	**2**	**1**	**0**	E
Affektuttryck	arg och glad ton				**1**	**0**	F
Perception av ansiktsuttryck	arg, glad, rädd/förvånad. Alla rätt=1, annars 0				**1**	**0**	F
Affektkontroll	poäng då FP svär mer än en gång, är högljudd, blir arg, gör sexuella kommentarer eller gester.				**1**	**0**	F
Spontan affekt	”Citron förbjuden”; visar munterhet=1				**1**	**0**	F
Fördröjd återgivning	– hus				**1**	**0**	E
	– träd				**1**	**0**	E
	– flyger				**1**	**0**	E
Medvetenhet vs utförande	Utfallet lika med prediktionen=1				**1**	**0**	G

Totalpoäng = _____ av 50

Kod	**BNI-screening: delskalor**	**Poäng**	
A	Tal- och språkfunktioner	_____	(15)
B	Orientering	_____	(3)
C	Uppmärksamhet/koncentration	_____	(3)
D	Visuospatial och visuell problemlösning	_____	(8)
E	Minne	_____	(7)
F	Affekt	_____	(4)
G	Medvetenhet om utförande	_____	(1)
Total delskalepoäng		_____	(41)

Totalpoäng = _____ Procent korrekt =_____