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SHORT REPORT

Evaluation of two plasma-based proteotyping assays against *APOE ε4* genotyping in a memory clinic setting: The Gothenburg H70 Clinical Studies

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Abstract

INTRODUCTION: Apolipoprotein E (*APOE*) ε4 allele status is associated with an increased risk of Alzheimer's disease and should be determined prior to initiation of anti-amyloid beta antibody treatment, because of increased risk of treatment-related side effects. Plasma-based apoE4 proteotyping may be an alternative to genotyping, with limited clinical evidence.

METHODS: apoE4 proteotyping was performed on 164 memory-clinic patients, using one chemiluminescent enzyme immunoassay (CLEIA) and one nucleic acid–linked immunosandwich assay (NULISA). The assays were evaluated against *APOE* ε4 blood genotyping.

RESULTS: The CLEIA had a 100% sensitivity and 98.5% specificity to classify *APOE* ε4 homozygosity and carriership in relation to genotyping. The NULISA had a 92.9% sensitivity and 97.1% specificity to classify homozygosity and a 100% sensitivity and 98.5% specificity to classify carriership.

DISCUSSION: The high performance suggests that the assays may be used as an easily available tool for identifying individuals for definitive *APOE* ε4 genotyping in a two-step approach.

KEYWORDS
Alzheimer's disease, apolipoprotein E ε4 genotyping, evaluation study, memory clinic, proteotyping

Highlights

- Plasma-based proteotyping presented good to excellent sensitivity in identifying apolipoprotein E (*APOE*) ε4 homozygosity.
- The negative predictive value was also very good to excellent, allowing us to rule out *APOE* ε4 homozygosity with high precision.
- Assays with excellent precision show potential for identifying individuals for definitive *APOE* ε4 genotyping in a two-step approach.

1 | INTRODUCTION

It has been estimated that dementia due to Alzheimer's disease (AD) affects 32 million individuals worldwide, and another 69 million have prodromal AD.[1] The disease is multifactorial and is associated with both environmental factors and genetic susceptibility. The ε4 variant of the apolipoprotein E (*APOE*) gene is the most established risk gene for sporadic AD, and individuals who are homozygous for the allele have a > 50% higher lifetime risk of developing AD, with a more rapid progression of disease.[2,3] In fact, recent results suggest that *APOE* ε4 homozygotes represent a genetic form of AD with close to 100% penetrance for cerebral β-amyloidosis.[4] These individuals are also at an increased risk of clinically relevant amyloid-related imaging abnormalities (ARIAs) and serious intracerebral hemorrhage by anti-amyloid antibody treatment.[5] Testing for *APOE* ε4 status should be performed prior to initiation of treatment to inform the risk of developing ARIAs. Prior to testing, prescribers should discuss with patients the risk of ARIAs across genotypes and the implications of genetic testing results.[6,7]

Previously, the traditional rationale for assessing *APOE* ε4 carriership in clinical practice has been to indicate risk and earlier onset of AD. The National Institute on Aging–Alzheimer's Association research framework does not formally include genetic markers, such as *APOE* ε4 status, when defining or staging AD. This is because these markers do not measure pathological changes but indicate an individual's future risk of pathological change.[8,9] However, models combining *APOE* ε4 status with blood-based markers of AD pathology, specifically plasma phosphorylated tau (p-tau)217, have recently been shown to predict AD with high precision.[10]

The gold standard for assessing *APOE* ε4 status is through blood-based genotyping.[11] However, this analysis is only available in laboratories with genotyping expertise. An alternative to direct genotyping is plasma-based proteotyping, in which measuring the apoE4 protein

isoform concentration provides information on the individual's underlying genotype. Early reports have indicated a concordance > 99% in unselected samples,[12] and the possibility of reliably determining *APOE* ε4 status through a plasma-based method may increase the availability to a larger group of individuals eligible for amyloid beta (Aβ)-targeting treatment.

Here, we evaluate two plasma-based apoE4 proteotyping assays against *APOE* ε4 blood genotyping, in a clinical sample of patients with cognitive symptoms from the Gothenburg Memory Clinic at Sahlgrenska University Hospital, the second largest university hospital in Sweden.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature primarily using PubMed. Few studies have previously evaluated plasma based proteotyping assays of apoE4, and studies in a clinical setting are currently missing.
2. **Interpretation**: Plasma-based proteotyping assays had an overall high precision, with the best performance in identifying apolipoprotein E (*APOE*) ε4 carriership and ruling out *APOE* ε4 homozygosity. As neither assay perfectly matched the gold standard of *APOE* ε4 genotyping, they show potential for use as easily available selection tools for identifying individuals for definitive *APOE* ε4 genotyping in a two-step approach.
3. **Future directions**: Clinical studies should replicate this study in other parts of the world to improve the generalizability of our observations. Furthermore, large replicative studies should also determine whether plasma-based proteotyping may be a sufficient method alone for detecting *APOE* ε4 homozygosity in patients under consideration for treatment with anti–amyloid beta antibodies in Alzheimer's disease.

2 | METHODS

2.1 | Study participants and sample collection

The study examines a subsample of participants in the H70 Clinical Studies, a clinical longitudinal observation study on individuals with memory complaints, enrolled at the Memory Clinic at the Sahlgrenska University Hospital in Gothenburg, Sweden. All patients referred to the Memory Clinic are invited to participate in the H70 Clinical Studies and are included on their first visit to the clinic. The study was conducted according to the Declaration of Helsinki approved by the Swedish Ethical Review Authority. All the participants and/or their close relatives gave written informed consent before any study-related procedures were performed. The study participants were examined by clinical staff and venous blood and plasma were all collected on the first visit. Global score of Clinical Dementia Rating (CDR) was used to classify participants as subjective cognitive decline (SCD, CDR = 0), mild cognitive impairment (MCI, CDR = 0.5), or dementia (CDR ≥ 1). Cerebrospinal fluid (CSF) was collected at a later visit, a few months later. Venous blood was centrifuged and stored at −80°C until analysis. CSF was collected by a physician at the Memory Clinic and were for analysis at the neurochemistry laboratory at the Sahlgrenska University Hospital within 40 minutes of sampling.

2.2 | *APOE ε4* genotyping and apoE4 proteotyping

APOE ε4 genotyping was performed from frozen blood with the TaqMan technique, at the Centre of Medical Genomics at the Sahlgrenska University Hospital.

apoE4 proteotyping was performed using two plasma-based assays, one adopting chemiluminescent enzyme immunoassay (CLEIA) technology, and the other adopting nucleic acid–linked immunosandwich assay (NULISA) technology. Both assays quantified the ratio of apoE4 to total apoE for classification of genotype. The CLEIA-based Lumipulse ApoE4 assay (#81453, Fujirebio) was used together with the Lumipulse G pan-apoE assay (#81449, Fujirebio) to classify participants into non-carriers (a ratio of apoE4/pan-apoE < 5%), heterozygotes (≥5%–75%), or homozygotes (≥ 75%) for *APOE* ε4. The cut-offs were provided by the manufacturer. The NULISA-based method also combined an apoE4 assay (prototype, Alamar), together with a pan-apoE assay (prototype, Alamar) to classify participants into non-carriers (a ratio of apoE4/apoE < 0.5%), heterozygotes (≥ 0.5%–15%), or homozygotes (> 15%) for *APOE* ε4. The cut-offs were set manually for optimal separation based on plotting the ratio for the three genotyping outcomes.

2.3 | Data analysis

The precision of using plasma-based proteotyping of apoE4/apoE assays for classification of *APOE* ε4 carriership status was compared to gold standard *APOE* ε4 genotyping. Primary outcome was classification of *APOE* ε4 homozygosity. Secondary outcomes were sex-stratified subgroup analysis, and *APOE* ε4 carriership (hetero- and homozygotes).

3 | RESULTS

3.1 | Study participants

In total, 164 individuals were included in this study, with an even proportion of men and women (81/83). Stratifying participants by *APOE* ε4 carriership through genotyping did not suggest any major influence on sex, age, or education (Table 1). As could be expected, non-carriers had milder cognitive status, with a higher proportion of individuals with MCI. Furthermore, the proportion of individuals with manifest

TABLE 1 Characteristics of study participants stratified by *APOE* ε4 genotype status ($n = 164$).

	Non-carrier ($n = 66$)	Heterozygote ($n = 70$)	Homozygote ($n = 28$)
Demographic variables			
Male sex	38 (58)	27 (39)	16 (57)
Age (years)	64 (9)	67 (8)	67 (7)
Education (years)	12 (3)	13 (4)	13 (3)
Comorbidities			
SCD	1 (2)	3 (4)	0 (0)
MCI	56 (85)	48 (69)	21 (75)
Dementia	9 (14)	19 (27)	7 (25)
CKD	7 (11)	7 (10)	3 (11)
Diabetes	17 (26)	2 (3)	3 (11)
CSF biomarkers			
Aβ42/40	0.08 (0.03)	0.06 (0.02)	0.04 (0.01)
p-tau (pg/mL)	51 (35)	65 (39)	89 (53)

Note: Data are presented as *n* (%) for categorical variables, and mean (SD) for continuous variables.
Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; CKD, chronic kidney disease; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; p-tau, phosphorylated tau; SCD, subjective cognitive decline; SD, standard deviation.

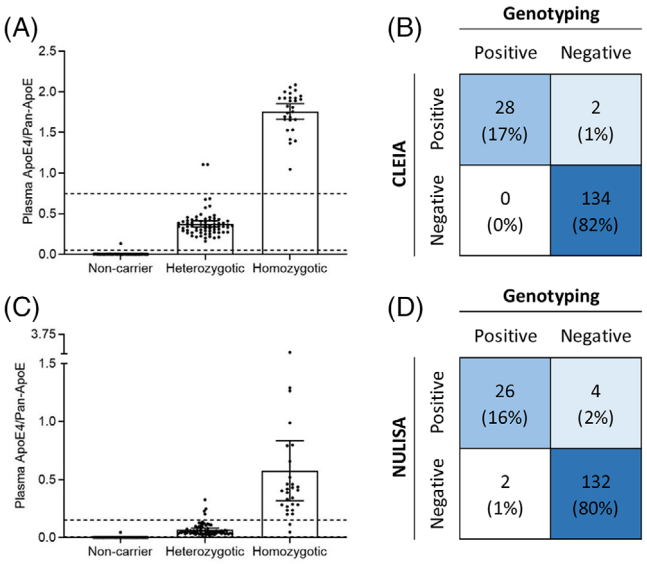

FIGURE 1 Ratio of plasma-apoE4/pan-apoE for participants stratified by *APOE* ε4 genotype status, with associated classification matrix for *APOE* ε4 homozygote (positive) or non-homozygote (negative) determined through CLEIA technology (A and B), as well as NULISA technology (C and D). Cut-offs for hetero- and homozygosity are marked as dashed lines. *APOE*, apolipoprotein E; CLEIA, chemiluminescent enzyme immunoassay; NULISA, nucleic acid–linked immunosandwich assay.

dementia was higher among participants homozygous for *APOE* ε4. CSF biomarkers also demonstrated a progressive shift by genotype, with the lowest Aβ42/40 ratio and the highest levels of p-tau among participants homozygous for *APOE* ε4.

3.2 | Precision of plasma-based proteotyping apoE4 assay against *APOE* ε4 genotyping

The CLEIA-based apoE4/pan-apoE ratio presented a very well separated distribution between participants stratified according to *APOE* ε4 status. A small number of individuals were outliers within each respective group, which resulted in overlaps among the three groups (Figure 1A and 1B). The NULISA-based method had a larger spread within each genotype, and a slightly higher overlap between the genotypes (Figure 1C and 1D).

The CLEIA-based assay had a 100% sensitivity for classifying both *APOE* ε4 homozygosity and carriership, with no difference between men and women (Table 2). The specificity was overall high, ≥ 98.5% for both the primary and secondary outcomes. The positive predictive value (PPV) was slightly lower in classification of *APOE* ε4 homozygosity for both men and women (ranging between 92.3% and 94.1%), but higher when used to classify *APOE* ε4 carriership (PPV: 99%). Negative predictive value (NPV) was 100% for all outcomes. *APOE* ε2 and ε3 variants were not detected by either assay, as can be seen among the *APOE* ε4 non-carriers (Figure 1A and 1C). In total, three individuals were differently classified than by genotyping. Two were genotyped as heterozygote but classified as homozygote and one was genotyped as a non-carrier, but classified as a heterozygote by proteotyping. However, these individuals all matched in classification between the CLEIA-based and NULISA-based assay.

TABLE 2 Accuracy of a CLEIA-based plasma-proteotyping apoE4 assay against *APOE* ε4 genotyping.

	Sensitivity	Specificity	PPV	NPV
Primary outcome				
APOE ε4 homozygosity	100	98.5	93.3	100
Secondary outcomes				
APOE ε4 homozygosity, males	100	98.5	94.1	100
APOE ε4 homozygosity, females	100	98.6	92.3	100
APOE ε4 carriership	100	98.5	99.0	100

Abbreviations: *APOE*, apolipoprotein E; CLEIA, chemiluminescent enzyme immunoassay; NPV, negative predictive value; PPV, positive predictive value.

The NULISA-based assay had a lower sensitivity (92.9%) than the CLEIA-based assay for classifying both *APOE* ε4 homozygosity, with similar results for men and women (Table 3). The assay had 100% sensitivity for identifying *APOE* ε4 carriership. The specificity was overall high, > 95% for both the primary and secondary outcomes, although not as high as for the CLEIA-based assay. As seen with the CLEIA-based assay, the PPV was slightly lower in classification of *APOE* ε4 homozygosity for both men and women (ranging between 84.6% and

TABLE 3 Accuracy of a NULISA-based plasma-proteotyping apoE4 assay against *APOE* ε4 genotyping.

	Sensitivity	Specificity	PPV	NPV
Primary outcome				
APOE ε4 homozygosity	92.9	97.1	86.7	98.5
Secondary outcomes				
APOE ε4 homozygosity, males	93.8	96.9	88.2	98.4
APOE ε4 homozygosity, females	91.7	97.2	84.6	98.6
APOE ε4 carriership	100	98.5	99.0	100

Abbreviations: *APOE*, apolipoprotein E; NPV, negative predictive value; NULISA, nucleic acid–linked immunosandwich assay; PPV, positive predictive value.

88.2%), but higher when used to classify *APOE* ε4 carriership (PPV: 99%). NPV was ≈ 98.5% for all outcomes except *APOE* ε4 carriership for which the NPV was 100%. In total, seven individuals were differently classified than by genotyping (three matching the CLEIA-based assay). Four were genotyped as heterozygote but classified as homozygote and two genotyped as homozygote but classified as heterozygote by proteotyping. One was genotyped as a non-carrier but classified as a heterozygote by proteotyping.

As the NULISA-based assay was a prototype, all samples deviating from the genotyping classification were retested along with some samples which were previously correctly classified by the assay. In the retest, samples were analyzed in duplicate instead of singlicate in the initial run. In the retest, all samples resulted in the same classification, except four out of the seven samples previously misclassified. These four samples changing classification had high concentrations on both pan-apoE and apoE4, which may have contributed to a higher variability in those samples. The samples that did not match with genotyping were the same samples misclassified by the CLEIA-based assay. Thus, after the retest, the results from the NULISA-based method were identical to the CLEIA-based assay but differed in three samples from the genotyping. We therefore performed a second genotyping on new blood aliquots from these three individuals, to rule out any technical problem in the genotyping. The reanalysis did not change genotype for any participant.

We also examined the performance of the assays exclusively in participants with pathological CSF Aβ42/40 and MCI or mild dementia (defined as CDR = 0.5–1), who may be eligible for Aβ antibody treatment (n = 98, characteristics in Table S1 in supporting information). As the CLEIA- and NULISA-based assays were identical after the second analysis run, the outcome was the same for the two assays in identifying participants homozygous for *APOE* ε4. The assays had a 100% sensitivity, a 98.6% specificity, a PPV of 96.4%, and a 100% NPV (classification matrix shown in Figure S1 in supporting information).

4 | DISCUSSION

We evaluated two plasma-based apoE4 proteotyping assays on predicting *APOE* ε4 genotype in a clinical cohort of 164 memory clinic patients with cognitive symptoms. The assays had some differences, but an overall high precision, with the best performance in identifying *APOE* ε4 carriership and ruling out *APOE* ε4 homozygosity. One of the two assays made no misclassifications, while the other had an NPV of 98.5%. The sensitivity was 100%, and the specificity was ≥ 98.5% for all outcomes in the CLEIA-based assay, and for the NULISA-based assay, sensitivities were ≥ 91.7% and specificities ≥ 96.9% for all outcomes.

Plasma-based proteotyping has previously demonstrated high precision in classifying small samples of unselected individuals by *APOE* ε4 status.[12–14] Here, we extend these reports by evaluating two assays in a clinical sample of 164 patients at a memory clinic, demonstrating equally high precision. With the approval of anti-Aβ antibodies for the treatment of AD, the clinical use of *APOE* ε4 testing has shifted from being a prognostic marker, to also being a safety marker indicating risk of potentially serious medical side effects. A large proportion of patients with AD may be eligible for treatment with anti-Aβ antibodies, which drastically will increase the need for *APOE* ε4 testing.[15] A plasma-based proteotyping analysis may be easier to implement in some laboratories for testing at a larger scale and may be less expensive.[16]

Plasma-based proteotyping increases the availability of *APOE* ε4 testing, which may lend itself to an array of uses. Alongside clinical use, there may be an interest to a wider public for determining an individual's AD risk. *APOE* ε4 testing has been publicly available for more than a decade, and while benefits of ruling out *APOE* ε4 carriership have been reported as a positive experience for individuals with a negative result, there are still ethical concerns for testing in a public domain.[17,18] In this study, the PPV of the two tests was lowest compared to the other outcome measures. The use of biomarkers in clinical use has been outlined by the US Food and Drug Administration, and its implications on AD have been discussed previously.[19] Here, the good to excellent sensitivity suggests that the major benefit of plasma-based proteotyping may lie in providing an increased availability for clinicians to identify individuals with a high likelihood of being *APOE* ε4 carriers, and rule out individuals who are not hetero- or homozygous. While proteotyping does not present a perfect PPV, neither test made any misclassification in ruling out non-carriers in our study. This suggests that DNA analysis may not be necessary for individuals eligible for Aβ-targeting treatment, if the proteotyping test is negative. DNA analysis may then be reserved for those individuals who tested positive for carriership, by applying a two-step approach.

There are some limitations to consider. Although plasma-based proteotyping shows a very high diagnostic accuracy, there are some variations between the different techniques evaluated in this study. There are also practical differences between the two assays, as the CLEIA-based assay measures pan-apoE and apoE4 in two separate runs. Although not clinically validated, the NULISA-based assay can measure both pan-apoE, apoE4, and 118 other markers (including Aβ40, Aβ42, and p-tau) in the same run.[12,14] Furthermore, neither test perfectly matched the current gold standard method of genotyping. As the sensitivity in this study was 100% for both assays on carriership, the plasma-based test could be an easily accessible method of identifying individuals with a high likelihood of being *APOE* ε4 carriers.

However, it should be noted that the study is limited in size and only included 28 *APOE* ε4 homozygous participants. For likely *APOE* ε4 carriers for whom specific status is necessary for initiation of Aβ-targeting treatment, genotyping can be used for a definitive determination in these selected individuals. In addition, as the negative predictive value was 100%, the CLEIA-based assay also appears to rule out *APOE* ε4 homozygosity with high reliability, and the NULISA prototype indicated a similar potential. Although genotyping is considered the gold standard for determination of *APOE* ε4 status, it is interesting to note that both assays classified three individuals similarly, but in discrepancy to the genotyping. It can therefore not be ruled out that there may be a misclassification in the assessed genotype in some cases. For example, it has previously been reported that discrepancies between genotyping and proteotyping can be caused by nonsense mutations in the *APOE* ε4 allele.[12] A limitation when proteotyping apoE4 specifically is that the analysis does not provide information on other genotypes, and information on the potentially protective *APOE* ε2 allele cannot be obtained. However, this information is not necessary in a clinical risk assessment when treating patients with anti-Aβ antibodies.

5 | RECOMMENDATIONS

1. Plasma-based proteotyping had an excellent sensitivity in identifying *APOE* ε4 homozygosity in a clinical sample of memory clinic patients. Additional clinical studies should replicate this study with larger sample sizes, preferably in other parts of the world to improve generalizability.
2. The NPV was also excellent with the commercially available assay, allowing us to rule out *APOE* ε4 homozygosity with high precision. Replicative studies of this observation should determine whether plasma-based proteotyping may be a sufficient method alone for detecting *APOE* ε4 homozygosity in patients under consideration for treatment with anti-Aβ antibodies in AD.
3. Both assays had an excellent sensitivity and NPV to identify potential *APOE* ε4 carriers. Additional studies should verify if plasma-based proteotyping may be used as a tool for identifying individuals for definitive *APOE* ε4 genotyping in a two-step approach.

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CONFLICT OF INTEREST STATEMENT

Anna Dittrich, Kübra Tan, Andrea L. Benedet, Ingmar Skoog, and Kina Höglund report no conflicts of interest relevant to the manuscript. Kaj Blennow has served as a consultant and on advisory boards for Abbvie, AC Immune, ALZpath, AriBio, Beckman-Coulter, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Neurimmune, Novartis, Ono Pharma, Prothena, Quanterix, Roche Diagnostics, Sanofi, and Siemens Healthineers; has served on data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials, and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai, and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this study. Silke Kern has served on scientific advisory boards, and as speaker and/or as consultant for Roche, Eli Lilly, Geras Solutions, Optoceutics, Biogen, Eisai, Merry Life, Triolab, Novo Nordisk, and Bioarctic, unrelated to present study content. Henrik Zetterberg has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZpath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, Roche, and WebMD, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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