# From Genotyping to Phenotyping: Optimizing APOE4 Carrier Classification Using ApoE4/Total ApoE Ratios

Sarah Auguste McCaulley[1], Isabelle Philipp[1], Lauren H. Johnson[1], Zuguang Wang[1], Suparna Ghanvatkar[2], Ankit Gupta[2], Marisa N. Denkinger[3], Alpana Singh[3], Taina M. Marques[3], Kathryn L. Ghisays[3], Robert J. Bauer III[4], Eric M. Reiman[4], Nicholas J. Ashton[3,4], Arejas J. Uzgiris[1]

[1]Clinical and Technology Innovation, Siemens Healthcare Diagnostics Inc, Walpole, MA, USA; [2]Clinical and Technology Innovation, Siemens Healthcare Diagnostics Inc, Bangalore, India; [3]Banner Sun Health Research Institute, Sun City, AZ USA; [4]Banner Alzheimer's Institute, Phoenix, AZ, USA

## Background

Apolipoprotein E (APOE) ϵ4 allele is the strongest common genetic risk factor for Alzheimer's disease (AD)[1]. Heterozygous carriers (ϵ2/ϵ4 or ϵ3/ϵ4) have an estimated 3–4-fold increased risk while homozygous carriers (ϵ4/ϵ4) have a 9–15-fold increased risk of developing AD[2]. APOE4 carrier status is also a critical determinant of ARIA risk associated with anti-amyloid therapies, with clinical trials reporting higher rates of symptomatic ARIA in homozygous carriers than in heterozygotes or non-carriers[3-5]. APOE genotyping is required prior to receiving anti-amyloid therapies in Europe and is increasingly recommended in other countries to support treatment selection and ARIA risk stratification[6].

However, transcriptional regulation and post-translational modifications associated with aging and disease may contribute to discordance between APOE genotype and circulating protein levels[7]. Phenotyping using blood-based assays provides a potential complementary approach that captures functional ApoE protein expression[8]. ApoE4 concentrations alone may prove insufficient to separate homozygotes and heterozygotes because plasma ApoE4 concentrations are influenced by interindividual factors such as lipid metabolism and diet[9]. Measurement of total ApoE levels can serve as an internal normalizer to reduce biological variability[10].

## Objective

We sought to investigate whether normalizing ApoE4 concentrations to total ApoE in a ratio improves agreement with genotyping for accurate classification of ApoE4 carrier status.

## Methods

Plasma samples from the Arizona APOE Cohort were analyzed on the Atellica® CI Analyzer using readily available ApoE4 and total ApoE assays*. Sample distribution by APOE genotype and the analytical workflow are shown in Figures 1 and 2. For cutoff optimization, numerical outliers were removed, a train-test split (80%/20%) was designed and metrics were calculated on the held-out test set.

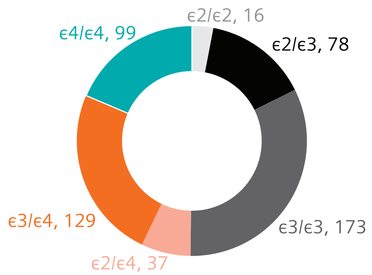


**Figure 1. Distribution of APOE Genotypes in the Arizona APOE cohort.** The pie chart shows the proportion of participants (N = 532) with each APOE genotype (16 ϵ2/ϵ2, 78 ϵ2/ϵ3, 173 ϵ3/ϵ3, 37 ϵ2/ϵ4, 129 ϵ3/ϵ4, and 99 ϵ4/ϵ4), illustrating the full genotype composition of the cohort.

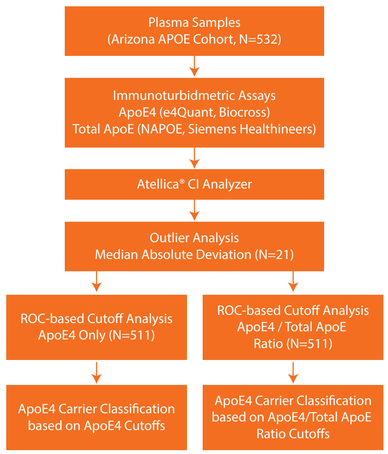


**Figure 2. Data generation and analysis workflow for ApoE4 and Total ApoE measurements and cutoff optimization**
Plasma samples were analyzed using readily available immunoturbidimetric assays for ApoE4 and total ApoE on the Atellica® CI Analyzer. Outliers were identified and removed using median absolute deviation (MAD) for cutoff optimization. ROC-based cutoffs were then derived using ApoE4 concentrations alone or the ratio of ApoE4 to total ApoE and applied for sample classification.

## Results

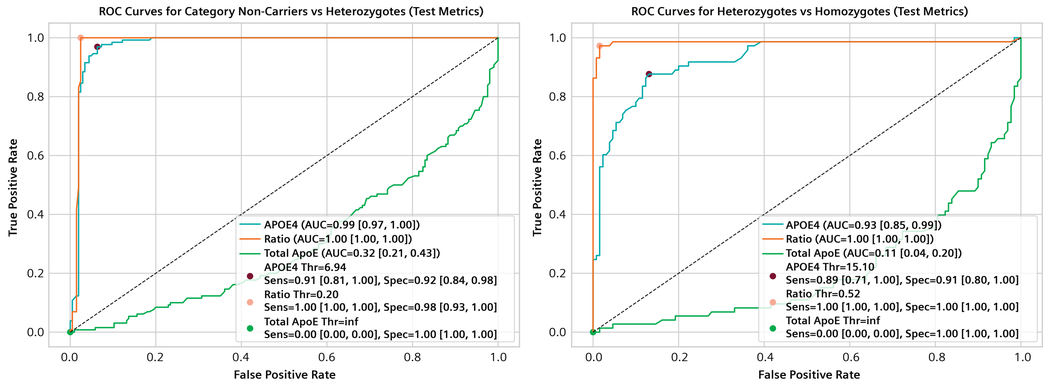


**Figure 3. Receiver operating characteristic (ROC) curves comparing the discriminative performance of ApoE4, total ApoE, and the ApoE4/total ApoE ratio measured by immunoturbidimetric assays.** Classification performance is summarized by the area under the curve (AUC). The ApoE4/total ApoE ratio demonstrates superior discriminative capability relative to ApoE4 alone, whereas total ApoE provides minimal discriminatory value. Thresholds were optimized to maximize Youden's J statistic (sensitivity + specificity – 1) and applied unchanged to a held-out test set. Test set performance is reported with 95% confidence intervals estimated using bootstrap resampling (reported in square brackets). Left panel: non carriers versus heterozygotes. Right panel: heterozygotes versus homozygotes.

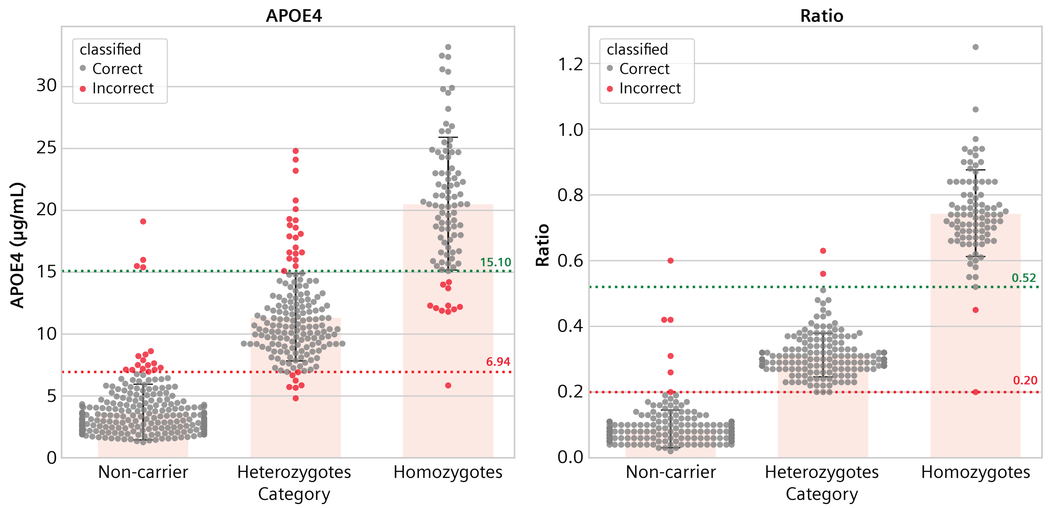


**Figure 4. Comparison of ApoE4 carrier classification using ApoE4 concentration and ApoE4/total ApoE ratio cutoffs.** Samples were classified as ApoE4 non-carriers, heterozygotes, or homozygotes using predefined thresholds from Figure 3, based on ApoE4 concentration and the ApoE4/total ApoE ratio. Dotted lines denote the thresholds (red: lower limit; green: upper limit). Categories are based on genotype. Each dot represents an individual sample, and bars indicate the mean ± SD. Samples misclassified using these thresholds are highlighted in red.

## Results

The ROC analysis demonstrated improved discriminatory performance when ApoE4 concentration was normalized to total ApoE (Figure 3). For distinguishing heterozygotes from non carriers, ApoE4 concentration alone achieved 91% sensitivity and 92% specificity at an optimal cutoff of 6.94 µg/mL. Normalization to total ApoE improved performance to 100% sensitivity and 98% specificity at an optimal cutoff of 0.20 (Figure 3, left).

Similarly, for differentiating homozygotes from heterozygotes, ApoE4 concentration alone yielded 89% sensitivity and 91% specificity at a cutoff of 15.10 µg/mL, whereas use of the ApoE4/total ApoE ratio achieved the highest discrimination with 100% sensitivity and specificity at a cutoff of 0.52 (Figure 3, right).

Statistical significance analysis indicated that ApoE4 and the ApoE4/total ApoE ratio support meaningful cutoff based classification, whereas total ApoE alone showed no discriminatory thresholds (Figure 3).

Comparison of ApoE4 carrier classification using ApoE4 concentration alone versus the ApoE4/total ApoE ratio demonstrated improved classification using the ratio based approach (Figure 4). Overall, both methods showed high agreement with APOE genotype; however, a small subset of samples (red dots) were misclassified when using ApoE4 concentration alone but were consistently and accurately classified when the ApoE4/total ApoE ratio was applied, indicating improved discrimination of carrier status with normalization to total ApoE. The samples that remained misclassified (red dots) with APOE genotype using the ratio based approach may reflect biological factors such as post translational modifications or altered ApoE4 detectability independent of genotype. Overall, incorporation of the ApoE4/total ApoE ratio reduced misclassification and improved accuracy of ApoE4 carrier classification compared with ApoE4 concentration alone.

## Conclusion

High-throughput ApoE4 phenotyping enables potentially accurate classification of APOE4 carrier status. Normalization to total ApoE markedly improves performance, minimizing misclassification among heterozygotes. These findings support ratio-based phenotyping as a robust and scalable alternative to genotyping for research applications.

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## References

1. Narasimhan S. *Nat Neurosci.* 2024 Jul;27(7):1236-1252.
2. Yamazaki Y. *Nat. Rev. Neurol.* 15, 501–518.
3. Budd Haeberlein S. *J Prev Alzheimers Dis.* 2022;9:197–210.
4. Van Dyck C. H. *N. Engl. J. Med.* 388, 9–21. doi: 10.1056/NEJMoa2212948
5. Sims J. R. *JAMA* 330, 512–527. doi: 10.1001/jama.2023.13239.
6. Rajič Bumber J. *J Clin Med.* 2025 Aug 26;14(17):6047. doi: 10.3390/jcm14176047.
7. Dupuy AM. *Clin Chem Lab Med.* 2001 May;39(5):405-13. doi: 10.1515/CCLM.2001.065.
8. Rezeli M. *EuPA Open Proteomics.* 2015;8:6.
9. Srivastava RAK. *Mol Cell Biochem.* 1996;155:153–62.
10. Yuri T. *Explor Neurosci.* 2023;2:238–44.

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