Featured Article

A genetics-based biomarker risk algorithm for predicting risk of Alzheimer’s disease

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Abstract

Introduction: A straightforward, reproducible blood-based test that predicts age-dependent risk of Alzheimer’s disease (AD) could be used as an enrichment tool for clinical development of therapies. This study evaluated the prognostic performance of a genetics-based biomarker risk algorithm (GBRA) established on a combination of apolipoprotein E (APOE)/translocase of outer mitochondrial membrane 40 homolog (TOMM40) genotypes and age, then compare it to cerebrospinal fluid (CSF) biomarkers, neuroimaging, and neurocognitive tests using data from two independent AD cohorts.

Methods: The GBRA was developed using data from the prospective Joseph and Kathleen Bryan, Alzheimer’s Disease Research Center study (n = 407; 86 conversion events [mild cognitive impairment {MCI} or late-onset Alzheimer’s disease {LOAD}]). The performance of the algorithm was tested using data from the Alzheimer’s Disease Neuroimaging Initiative study (n = 660; 457 individuals categorized as MCI or LOAD).

Results: The positive predictive values and negative predictive values of the GBRA are in the range of 70%–80%. The relatively high odds ratio (approximately 3–5) and significant net reclassification index scores comparing the GBRA to a version based on APOE and age alone support the value of the GBRA in risk prediction for MCI due to LOAD. Performance of the GBRA compares favorably with CSF and imaging (functional magnetic resonance imaging) biomarkers. In addition, the GBRA “high” and “low” AD-risk categorizations correlated well with pathologic CSF biomarker levels, positron emission tomography amyloid burden, and neurocognitive scores.

Discussion: Unlike dynamic markers (i.e., imaging, protein, or lipid markers) that may be influenced by factors unrelated to disease, genomic DNA is easily collected, stable, and the technical methods for measurement are robust, inexpensive, and widely available. The performance characteristics of the GBRA support its use as a pharmacogenetic enrichment tool for LOAD delay-of-onset clinical trials and merit further evaluation for its clinical utility in evaluating therapeutic efficacy.

1Data used in preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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It can take decades of undetected disease progression before frank symptoms of cognitive decline are diagnosed in Alzheimer’s disease (AD) patients. Treatments that delay or even prevent AD dementia require robust prognostic biomarkers of the preclinical disease process for accurate patient selection into clinical trials. Effective biomarkers with reproducible performance characteristics that are relatively inexpensive can be used for enrichment of prevention trial cohorts and, perhaps, for subsequent identification of individuals most suitable for intervention.

To date, cerebrospinal fluid (CSF, amyloid-beta [Aβ]_1–42, total tau [t-tau], and phosphorylated tau [p-tau]) and neuroimaging biomarkers (structural/functional magnetic resonance imaging [fMRI] and amyloid-imaging) have been among the most studied biomarkers in individuals with prodromal AD symptoms (mild cognitive impairment [MCI]) or full AD dementia. These biomarker methods are currently the “gold standard” for biomarker-based risk prediction and their clinical utility is described in opinions of the International Working Group (IWG), National Institute of Aging-Alzheimer’s Association (NIA-AA), European Medicines Agency (EMA), and Food and Drug Administration (FDA) [1–4]. However, CSF biomarkers suffer from the invasive nature of a lumbar puncture, issues with laboratory-to-laboratory variability and reproducibility, and a lack of globally recognized reference standards and cutoff values. In addition, neuroimaging methods require (1) specialized, expensive magnetic resonance imaging (MRI) and/or positron emission tomography (PET) scanning equipment that are only available at specific medical centers; (2) use of labile reagents; (3) specially trained medical personnel to administer the tests and interpret the results [5]; and (4) establishment of threshold/cutoff values meaningful for clinical observations.

Blood-based biomarkers have the potential to be easier to obtain and more economical; platforms to test these are widely available at medical facilities around the world. Unfortunately, there are multiple factors that can confound the measurement of RNA, protein, and/or metabolite levels in the blood and correlation with AD disease state, including diseases comorbid with AD, various medical treatments, and even diet. Strong prognostic biomarkers that can predict future onset of AD would ideally be dichotomous (marker positive/negative) and not continuously variable, i.e., where different analyte levels correspond to different risks and assignment of arbitrary cutoff values are imposed.

A simple, genetics-based biomarker risk algorithm (GBRA) using a combination of apolipoprotein E (APOE, ε2,ε3,ε4), translocase of outer mitochondrial membrane 40 homolog (TOMM40) rs10524523 variable length poly-T repeat polymorphism (TOMM40/523) genotypes, and age has been developed as a prognostic tool for assessing AD age-of-onset (AOO) in asymptomatic people [6]. In this study, we present data on the predictive characteristics of the GBRA to identify people at risk for MCI due to AD [7], and comparative data for CSF and neuroimaging (fMRI) based biomarkers, and neurocognitive testing. The overall hypothesis to be tested is that the combination of age, APOE genotype, and TOMM40/523 genotype, used in an algorithm based on historical MCI/AD AOO data, will outperform algorithms based on age alone or APOE genotype in predicting conversion from normal cognition to dementia (phenoconversion) when assessed by receiver operating curves (ROC) analysis or other well-defined statistical methods to compare biomarkers. Also compared are the categories for risk of phenoconversion with widely used biomarkers for AD including CSF-based biomarkers, Pittsburgh Compound B (PIB)-PET imaging of amyloid burden, and neurocognitive tests.

2. Methods

2.1. AD cohorts

The Joseph and Kathleen Bryan, Alzheimer’s Disease Research Center (Bryan-ADRC) Memory, Health and Aging Study (MHA) cohort measured age of AD onset of subjects followed at the Bryan-ADRC at Duke University [8]. MHA participants included MCI patients from the Duke Memory Disorders Clinic and individuals who were enrolled in the Bryan-ADRC autopsy program as controls; some of these individuals have been followed for 10–20 years. The latter individuals were cognitively normal when they enrolled and many have progressed to AD or MCI. Study subjects were followed annually with the National Alzheimer’s Coordinating Center-Unified Data Set (NACC-UDS) protocol and battery [9] of neuropsychological tests to monitor cognitive changes and diagnose onset of cognitive impairment and probable AD dementia. Importantly, the subjects were followed prospectively to capture the earliest clinical symptoms of the disease process and were all assessed using validated tests including the NACC-UDS, along with standardized practices and definitions of symptom onset and cognitive status at one research center, the Bryan-ADRC. Cases of
MCI were diagnosed by a neurologist and neuropsychologist for individuals considered to be clinically in the prodromal stage of AD [10]. Details on the identification of prodromal AD in population settings are described in Mayeux et al. [11]. These criteria are the basis of the criteria for MCI due to AD used for the development of the GBRA and for the TOMORROW clinical trial [6,12]. Some of the cases were eventually autopsied to confirm the AD diagnosis.

For this analysis, the Alzheimer’s Disease Neuroimaging Initiative (ADNI, ClinicalTrials.gov identifier, NCT00106899) cohort refers to data from the ADNI-1 study conducted at multiple sites within North America with retrospective assessment of imaging (MRI and PET) and biomarkers from cognitively normal controls, MCI subjects defined by the ADNI protocol included subjects with MMSE scores >23, isolated memory impairment based on education adjusted memory scores on the Wechsler Memory Scale Logical Memory III scale used by ADNI and a clinical dementia rating (CDR) [13] global score of 0.5. Study participants with AD were diagnosed using standard criteria (NINCDS/ADRDA criteria for probable AD) [14] and were of mild severity at enrollment, with MMSE scores between 20 and 26 and CDR scores between 0.5 and 1.0. For evaluation of the performance of the GBRA to predict conversion from normal cognition to MCI and/or AD, data from healthy controls (HCs) who converted to MCI or AD were used to identify AOO. Furthermore, for AD participants, the best estimate of year of onset of symptoms (collected at screening) was used. Data for this cohort were obtained from the ADNI database (adni.loni.usc.edu). Data were downloaded on January 28, 2014 and included TOMM40 rs10524523 (TOMM40.csv), APOE (APOERES.csv), CSF biomarkers (UPENNBIOMK*.csv), PET imaging (PIBPET-SUVR.csv), and neuropsychological tests (CDR.csv, MMSE.csv). The entire ADNI-1 cohort (n = 660) that had complete records for APOE, TOMM40/523, and neurocognitive tests (MMSE and CDR) was used for the primary analysis; 332 subjects had CSF biomarker data and 66 had PET data available. The ADNI was launched in 2003 as a public-private partnership, led by principal investigator Michael W. Weiner. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see www.adni.info.org.

Clinical data from only Caucasian subjects of both the Bryan-ADRC and ADNI cohorts were used in this investigation.

2.2. Development of the GBRA

The GBRA was previously developed to enrich a clinical trial with subjects at an elevated near-term risk for onset of cognitive decline to evaluate efficacy of a therapeutic; details of the development of the GRBA are provided in the study by Crenshaw et al. [6]. In brief, the algorithm incorporates an individual’s current age along with TOMM40/523 and APOE genotypes (Fig. 1). This figure summarizes the risk stratification scheme that implements the GBRA. The low-risk stratum includes carriers of APOE e2/e2 and APOE e2/e3 and a proportion of APOE e3/e3 subjects. TOMM40/523 long (L/L) (i.e., APOE e4/e4 in white Caucasians) subjects and those with very long (VL)/L, which corresponds to an earlier APOE ε3/e4 AOO distribution, are classified as high risk. There are three common genotypes with risks that change as a function of age, where the high risk versus low risk distinction is determined by location of the age where the fraction of the cohort without cognitive impairment starts to decline rapidly from a level of approximately 80%. Individuals with the TOMM40/523 short (S)/L genotype, one of the two APOE e3/e4 groups, become high risk at age 75 years; 523 S/S subjects (a subgroup of the APOE e3/e3 subjects) enter the high-risk category at age 78; and 523 S/LV (a subgroup of the APOE e3/e3 subjects) become high risk at age 77. TOMM40/523 S/S–APOE e2/e4 subjects are included in the high-risk stratum as a consequence of carriage of an APOE e4–L haplotype (note that <3% of Caucasians possess this genotype). TOMM40/523 VL/LV subjects that correspond to the oldest APOE e3/e3 AOO distribution are classified as low risk.

Fig. 2 illustrates the relationships further using Kaplan-Meier (KM) curves showing the clear relationship between the APOE and TOMM40/523 genotypes in Caucasians that is based in the linkage disequilibrium between the variants and specifically how TOMM40/523 increases the precision of AOO estimation for the Bryan-ADRC cohort. In Caucasians, the APOE e4 allele is almost always (>98% of the time) linked to a TOMM40/523 “L” allele. Therefore, in this case, the two alleles are interchangeable for genotype/phenotype association (e.g., the APOE e4/e4 and TOMM40/523 L/L curves would be identical and TOMM40/523 does not contribute additional information to AOO.) APOE e3 and APOE e2 are linked to TOMM40/523 S or VL alleles and contribute information to the APOE e3/e4 and e3/e3 AOO curves as shown. APOE e2 allele linkage to TOMM40/523 alleles occurs in equivalent ratios to APOE e3 alleles and, therefore, the differentiation of the different AOO curves shown in Fig. 2 is not a consequence of APOE e2 carriage in opposition to APOE e3 but instead a consequence of the different TOMM40/523 alleles. As shown in Fig. 2, the major changes in slope of most of the KM curves for late-onset Alzheimer’s disease (LOAD) AOO occur between ages 67 and 83 years. After age 83, the risk for development of cognitive impairment is high for people of all genotypes except those carrying APOE e2/e2 or APOE e2/e3. The strong age by TOMM40/523 interaction and informativeness of the combination of APOE and TOMM40/523 genotypes observed in Fig. 2 is the basis for the GBRA.
2.3. APOE and TOMM40’523 genotyping

Samples were genotyped for APOE and TOMM40’523 at Polymorphic DNA Technologies (PolymorphicDNA.com, Alameda, CA, USA). The APOE genotype was defined by the rs429358 and rs7412 SNPs. The homopolymer poly-T rs10524523 was genotyped as described elsewhere [6,15]. Alleles of the rs10524523 variable length poly-T repeat polymorphism variant (TOMM40’523) were classified as described in the literature [6], according to the length of the poly-T repeats: S (<21T), L (21–29T), and VL (>29T). For quality control purposes, each sample was genotyped twice. The consensus calls of the TOMM40’523 genotypes based on the replicate assays were used for the genetic analysis.

2.4. Statistical analysis

Assessment of the performance of the GBRA is challenging due to the heterogeneity of the clinical diagnoses of LOAD and MCI [16]. As noted for each analysis of algorithm performance, clinical diagnoses of an event included either MCI or LOAD. Standard measures of predictive performance (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) are reported in addition to the odds ratio for the occurrence of a specified event(s) within a 5-year interval. Likelihood ratios are reported, weighted by prevalence for positive prediction (+) and negative prediction (−). Ninety-five percent confidence intervals (CIs) are reported for each performance measure.

Fig. 1. GBRA AD-risk algorithm. Flowchart and Tables show the process for the generation of the risk assessment for MCI due to AD using the GBRA. Risk of high or low is assigned based on APOE genotype, TOMM40’523 genotype, and current age. Abbreviations: GBRA, genetics-based biomarker risk algorithm; AD, Alzheimer’s disease; APOE, apolipoprotein E; TOMM40, translocase of outer mitochondrial membrane 40 homolog; MCI, mild cognitive impairment.
Several analyses are performed to compare the performance characteristics of alternate risk models composed of different measures, e.g., genetics, biochemical markers and clinical data (e.g., age) and different parameter settings (e.g. age thresholds) for the GBRA. Receiver operating curves (ROC) are provided which compare the GBRA to forms of the risk model based on age alone, APOE genotype alone, and the combination of age and APOE ε4 carrier status (carriage of at least one APOE ε4 allele). Logistic regression modeling and step-wise logistic regression modeling were performed to compare alternate forms of the risk model: reporting P values for each term, r² values, area under the curve (AUC), and Bayesian information criterion. To assess the sensitivity of the GBRA to changes in the age thresholds, all age-dependent thresholds (as shown in Fig. 1) were increased or decreased by 2 and 4 years and the resulting sensitivity and specificity were plotted. To provide a statistical measure of improvement of the GBRA with the TOMM40/523 genotype in comparison with a model based on APOE genotype and age alone, the net reclassification index (NRI) was calculated for both cohorts [17] and is reported with the corresponding P value. The index quantifies accurate changes in categories based on events (subject develops MCI or AD) or nonevents (subjects remain cognitively normal) comparing the two versions of the risk algorithm. Statistically significant improvement is observed if subjects move to the correct classification with the full version of the model and the NRI is compared with a null hypothesis of no improvement. Measures of sensitivity and specificity for CSF related and imaging biomarkers from multiple studies as reported in the qualification opinions by the European Medicines Agency (EMA) [2,4] are used for comparison with the GBRA.

For analysis of risk assessment by the GBRA in comparison with neurocognitive scores, CSF biomarkers and PET amyloid burden and APOE, TOMM40/523 genotype, and age at diagnostic assessment were used to classify risk for MCI due to AD as low or high based on the GBRA. For biomarkers that are expressed on an interval scale (CSF biomarkers (A β 1–42 and p-tau), PET imaging measures of A β load (standardized uptake value ratio, SUVR), and MMSE), a t test was performed to compare the mean levels of each biomarker between the two risk groups. P values <.05 were considered significant. A Tukey-Kramer honestly significant difference test was done to account for multiple comparisons when comparing the six TOMM40/523 genotypes with PET imaging markers of A β load.

All statistical analyses were performed using SAS (version 9.3; SAS Institute, Cary NC, USA) or JMP Genomics (version 6.0; SAS Institute).

3. Results

The Bryan-ADRC cohort was used to develop the GBRA; the ADNI cohort was used to assess the performance of the GBRA in an independent data set. Presented here are preliminary data on the predictive value of a GBRA based on age and two genetic variants: APOE genotype and TOMM40/523 genotype. The performance characteristics for the GBRA are compared with the performance characteristics of a CSF biomarker based on
the combination of Aβ1–42 and t-tau, which provided a PPV of 65% (53–77%, 95% CI) in a recent meta-analysis [2]. These CSF markers were recently qualified by the EMA for selecting patients for clinical trials of AD therapeutics [2].

3.1. Cohort characteristics

Table 1 summarizes the characteristics of the discovery (Bryan-ADRC) and replication (ADNI) cohorts. For each of the three diagnostic criteria (healthy controls (HCs), MCI, and AD), the demographics, global measures of cognitive function, and disease severity (MMSE and CDR score) are reported. The mean age of subjects (78–79 years) diagnosed with MCI and AD did not differ between the cohorts or between the diagnostic groups. The ages of the cognitively normal subjects in the Bryan-ADRC cohort (mean age of 72 years) were significantly different (P < .0001) from the ADNI cohort (mean age of 76 years). The frequency of APOE ε4 carriage did not differ significantly (P = .11, Fisher’s exact test) between the two cohorts. The MMSE scores for the AD diagnostic category for the Bryan-ADRC cohort were significantly lower than those for the ADNI cohort (P < .0001). There was a higher prevalence of MCI and AD in the ADNI cohort (69%) compared with that in the Bryan-ADRC cohort (40%).

3.2. Performance of the GBRA

Table 2 summarizes the performance characteristics of the GBRA for prediction of risk of MCI or AD in the two cohorts. The performance of the GBRA for prediction of AD risk was similar within the two patient cohorts for sensitivity (0.60 vs. 0.65; Bryan-ADRC vs. ADNI) but showed greater specificity for the Bryan-ADRC patients (0.81) than the ADNI patients (0.61). For the Bryan-ADRC cohort, a reasonable balance between PPV and NPV is obtained at 68% and 76%, respectively. For the ADNI cohort, PPV is relatively high at 79%, whereas NPV is calculated to be 44%.

3.3. Comparison of alternate forms of the GBRA

Fig. 3 shows the sensitivity and specificity of the GBRA in comparison with structural forms that include only age, APOE genotype, and APOE genotype and age. The full version of the algorithm includes age, APOE genotype, and TOMM40α523 genotype and offers an appropriate balance between sensitivity and specificity in comparison with forms of the algorithm that do not include TOMM40α523 genotype, notably to gain specificity at the expense of some sensitivity. For the ADNI cohort, the highest sensitivity is obtained using subset models that contain only age or age and APOE ε4 carrier status; however, the

Table 1

| Bryan-ADRC and ADNI cohort characteristics |
|-------------------------------------------|
| Characteristics                        | Healthy control subjects | Mild cognitive impairment | Alzheimer’s disease |
| ADNI                                     |                           |                           |                     |
| No. at baseline (No. at 5 y)             | 205 (203)                 | 304 (178)                 | 151 (279)           |
| Age, mean (SD), y                       | 76 (5)                    | 78 (5)*                   | 78 (5)              |
| Male: female ratio                      | 108:97                    | 202:102                   | 80:71               |
| APOE ε4 allele carrier, %               | 26.3                      | 52.6                      | 64.9                |
| Mini-mental state examination score, mean (SD) (MMSE) | 29.1 (1.0)              | 27.0 (1.8)                | 23.5 (2.1)          |
| Cognitive dementia rating scale global score (n) (CDR) | 0                         | 0.5                       | 0.5 (82) 1.0 (69)   |
| Cognitive dementia rating scale sum of boxes (CDR-SB), mean (SD) | 0.03 (0.12)              | 1.61 (0.90)               | 4.14 (1.63)         |
| Cerebrospinal fluid Aβ1–42 level at baseline, mean (SD) pg/mL, n | 204 (32) 102             | 162 (55) 153             | 146 (45) 77         |
| Cerebrospinal fluid p-tau level at baseline, mean (SD) pg/mL, n | 25.4 (15) 101            | 36.2 (18.8) 153          | 39.7 (17.8) 77      |
| PET index of amyloid burden at 1 y, (standardized uptake value ratio, SUVR) | 1.49 (0.32) 18       | 1.77 (0.41) 26             | 1.94 (0.36) 22     |
| Bryan-ADRC                              |                           |                           |                     |
| n                                        | 242                       | 79                        | 86                  |
| Age, mean (SD), y                       | 72 (9)                    | 79 (9)**                   | 78 (8)**            |
| Male: female ratio                      | 115:191                   | 38:58                      | 42.64               |
| APOE ε4 allele carrier, %               | 34.3                      | 43.8                       | 56.7                |
| Mini-mental state examination score, mean (SD) (MMSE) | 29.2 (1.1)              | 27.7 (2.3)                | 22.1 (6.8)          |
| Cognitive dementia rating scale global score (n) (CDR) | 0 (220) 0.5 (21)         | 0 (21) 0.5 (57) 1 (1)     | 0 (2) 0.5 (35) 1.0 (27) |
| Cognitive dementia rating scale sum of boxes (CDR-SB), mean (SD) | 0.09 (0.32)              | 1.07 (0.90)               | 5.92 (5.55)         |
| Cohort comparisons                      |                           |                           |                     |
| Age, P value                            | <.0001                    | .44                       | .51                 |
| MMSE, P value                           | .56                       | .0081                      | .02                 |
| CDR global score, P value               | <.0001                    | <.0001                     | <.0001              |
| CDR sum of boxes, P value               | .0044                     | <.0001                     | .0021               |

Abbreviations: SD, standard deviation; AD, Alzheimer’s disease; MCI, mild cognitive impairment; Bryan-ADRC, Joseph and Kathleen Bryan, Alzheimer’s Disease Research Center; ADNI, Alzheimer’s Disease Neuroimaging Initiative. 

NOTE. For comparison of age between diagnostic categories, *P < .05; **P < .001. Comparisons are to the healthy controls. There was no significant difference in age between the MCI and AD categories for either cohort.
specificity is low. The highest specificity is obtained from the subset of models based on APOE genotype alone; however, this increased specificity comes at the expense of reduced sensitivity. The performance of the algorithm differs between the two cohorts in terms of specific values of sensitivity and specificity; however, the same relative comparison of sensitivity and specificity is observed for the comparison of the subset models to the full GBRA.

The specific age thresholds that are used in the GBRA correspond to the location on each TOMM40/523 KM curve where the fraction of the Bryan-ADRC cohort without cognitive impairment starts to decline rapidly from a level of approximately 80%. Including these age-dependent thresholds has a statistically significant effect on the performance of the GBRA as assessed by changes in AUC. Inclusion of the thresholds improves AUC for both the Bryan-ADRC cohort (Figs. 4A and B) and Table 3 provide analytical data on the sensitivity and specificity of several models for prediction of conversion from cognitively normal to dementia (MCI or AD) in a 5-year time period. Statistical comparisons of the models are for contrasts of the AUC. The simplest model includes only age. Two models include terms for each genotype (APOE and ‘523) individually plus age. The most complex model includes age and the two genotypes. The GBRA is implemented as shown in Fig. 1. For the GBRA, sensitivity can be improved at the expense of specificity by lowering the age threshold (Fig. 5) and for the ROC analysis, age is varied to show the trade-off between sensitivity and specificity. For the Bryan-ADRC cohort (Fig. 4A), there is a notable, modest but not statistically significant improvement of the full model containing age, APOE genotype, and TOMM40/523 genotype in comparison with age or age combined with either of the two genotypes. For the ADNI cohort (Fig. 4B), the two genotypes either individually or together plus age give relatively the same ROC curves that are substantially better than age alone (P < .0001). For both cohorts, the combination of APOE and age in comparison with the combination of age and TOMM40/523 provides slightly better performance, although not statistically different. Area under the curve (AUC) values for the ADNI cohort are consistently lower than AUC values for the Bryan-ADRC cohort. The AUC for the GBRA is not statistically different from the combination of APOE and age for either cohort.

An alternate statistical framework to test for improvement of the GBRA with the TOMM40/523 genotype in comparison with a model based on APOE genotype and age alone, the NRI was calculated for both cohorts [17]. The NRI was highly significant (P = .0001) for the ADNI cohort but was not significant (P = .09) for the Bryan-ADRC cohort (Table 2). A recent critical review of NRIs for evaluation of risk prediction has identified several advantages and disadvantages (notably, taking an unweighted average of the reclassification categories to produce the index) of the method [18]. After the recommendation of this review, we have reported all reclassification categories in Table 2 and have supplemented the NRI analysis with a full description of the operating characteristics of the alternate risk models in Table 3 and Fig. 4.

### 3.4. Age sensitivity of the GBRA

The sensitivity and specificity of the GBRA depends, in part, on the age thresholds that are set in the algorithm. These thresholds were set based on the KM analysis of the Bryan-ADRC cohort with respect to development of MCI or AD by identifying the age where the slope of the KM curve changes most rapidly, starting at approximately 20% affected (subjects with MCI or AD). The age thresholds that are used in the algorithm provided optimal stratification of low- and high-risk subjects for the cohort. However, changing the age threshold affects both sensitivity and selectivity. Fig. 5 shows the trade-off in sensitivity and selectivity for the GBRA predictions for both the Bryan-ADRC and ADNI cohorts when the GBRA age thresholds are increased or decreased in intervals of 2 or 4 years. This analysis also shows the sensitivity of the algorithm to changes in the age thresholds.

### Table 2

| Statistic                  | Bryan-ADRC study | ADNI study |
|---------------------------|------------------|------------|
| PPV                       | 68 (60–74)       | 79 (74–83) |
| NPV                       | 76 (71–80)       | 44 (38–50) |
| Prevalence                | 0.40 (0.36–0.44) | 0.69 (0.66–0.73) |
| Sensitivity               | 0.60 (0.53–0.67) | 0.65 (0.61–0.70) |
| Specificity               | 0.81 (0.76–0.85) | 0.61 (0.53–0.67) |
| OR                        | 4.9 (3.4–7.3)    | 2.9 (2.1–4.1) |
| NRI Z score (with APOE and age) | 1.7             | 3.8             |
| NRI P                     | 0.09             | .0001         |
| NRI events reclassified up (true positives) | 0.07             | 0.04           |
| NRI events reclassified down (false positives) | 0.25             | 0.25           |
| NRI nonevents reclassified up (false negatives) | 0.05             | 0.01           |
| NRI nonevents reclassified down (true negatives) | 0.32             | 0.42           |
| LR (+)                    | 2.10 (1.66–2.66) | 3.74 (3.06–4.57) |
| LR (−)                    | 0.32 (0.27–0.39) | 1.28 (1.15–1.44) |

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio for development of MCI or LOAD; MCI, mild cognitive impairment; LOAD, late-onset Alzheimer’s disease; NRI, net reclassification improvement (Z score); APOE, apolipoprotein E; Bryan-ADRC, Joseph and Kathleen Bryan, Alzheimer’s Disease Research Center; ADNI, Alzheimer’s Disease Neuroimaging Initiative; LR, likelihood ratio, weighted by prevalence for positive prediction (+) and negative prediction (−).

NOTE: Values are shown with 95% confidence limits. NRI reclassification numbers are given as the proportion of events reclassified (events classified up are improved prediction, nonevents reclassified down are improved prediction).
3.5. Performance comparison of the GBRA with alternate biomarkers

The sensitivity and specificity of the GBRA compares favorably with CSF and imaging (fMRI) biomarkers measured in the ADNI cohort and recently qualified for clinical trials research by the EMA as shown in Fig. 6. Although there are reports of several biomarkers based on Aβ1-42 and t-tau that show improved sensitivity and specificity in comparison with the GBRA, there is considerable variability in the measurement of these biomarkers across clinical centers, based on multiple literature reports as shown in Fig. 6 [2,19–26].

3.6. Correlation of risk assessment by the GBRA with neurocognitive scores, CSF biomarkers, and PET amyloid burden

3.6.1. Neurocognitive scores

For the Bryan-ADRC cohort, subjects predicted to be low risk by the GBRA showed a significantly (P < .0001) higher mean MMSE score (28.37 ± 0.24) in comparison with subjects predicted to be high risk (26.10 ± 0.29; Table 4). This pattern was also observed in the ADNI cohort with high-risk subjects having a significantly (P < .0001) lower mean score (26.45 ± 0.13) compared with the mean score for low-risk subjects (27.37 ± 0.16). For reference, the difference in mean MMSE scores for HC and MCI subjects in the ADNI cohort is 2.1, whereas the difference between HC and AD subjects is 5.6. The difference in mean MMSE scores for HCs and MCI subjects in the Bryan-ADRC cohort is 1.5, whereas the difference between HC and AD subjects is 7.1. Highly statistically significant (P < .0001) differences in CDR global and CDR sum of boxes scores are observed between the high- and low-risk groups for both the Bryan-ADRC and the ADNI cohorts (Table 4). These results demonstrate that the GBRA correctly stratifies subjects by neurocognitive performance.

3.6.2. CSF biomarkers (Aβ1-42 and p-tau)

For the ADNI cohort, subjects predicted to be high risk by the GBRA showed a significantly (P < .0001) lower mean level of CSF Aβ1-42 (157.15 ± 3.78) in comparison with subjects predicted to be low risk (195.59 ± 4.67; Table 4). For reference, the difference in mean Aβ1-42 levels for HC and MCI subjects is 42, whereas the difference between HC and AD subjects is 58. Subjects predicted to be high risk by the GBRA showed a significantly (P < .0001) higher mean level of p-tau (36.70 ± 1.28) in comparison with subjects predicted to be low risk (29.17 ± 1.58). The difference in mean p-tau for HC and MCI subjects is 10.8, whereas the difference between HC and AD subjects is 14.3. These results show that the GBRA risk categories of high and low are associated with
relative differences in CSF levels of Aβ1–42 and p-tau generally observed in HC and MCI or AD patients (e.g., lower CSF Aβ1–42 and higher p-tau).

3.6.3. PET imaging measures of Aβ load

A marked difference is observed in PET imaging measures of Aβ load (standardized uptake value ratio, SUVR) between high- and low-risk subjects for the ADNI cohort (Table 4). Subjects predicted to be high risk by the GBRA showed a significantly (P < .0001) higher (worse) standardized uptake value ratio (SUVR) measurement of amyloid burden (mean SUVR 1.90 ± 0.06, n = 40) in comparison with subjects predicted to be low risk (mean SUVR 1.52 ± 0.07, n = 30). For reference, mean SUVR for HC subjects is 1.49 ± 0.08, mean SUVR for MCI subjects is 1.76 ± 0.08, and mean SUVR for AD subjects is 1.91 ± 0.08. The difference in mean SUVR for HC and MCI subjects is 0.27, whereas the difference between HC and AD subjects is 0.42.

4. Discussion

We evaluated a classification of risk as high or low, based on the likelihood of phenoconversion to MCI or AD within a 5-year time frame, typical for a delay-of-onset clinical trial, based on age, APOE genotype, and TOMM40/523 genotype. A retrospective analysis of the GBRA in two cohorts with a relatively high prevalence of phenoconversion from normal cognition to MCI or AD (40% Bryan-ADRC cohort, 69% ADNI cohort) is a suitable study to determine the correlation between risk categories determined by the GBRA and blood-, CSF-, and imaging-based biomarkers. For both cohorts, there was a strong correlation between the risk categories determined by the GBRA and neurocognitive measures (MMSE and CDR). For the ADNI cohort, where extensive data were available for CSF (Aβ1–42 and p-tau) and imaging biomarkers, a similar strong correlation between risk categories and these biomarkers of
neuropathologic findings was observed where the direction and magnitude of the correlation was consistent with previous publications which compared the biomarker to diagnoses of MCI, AD, or HC [27].

The performance of the GBRA for prediction of AD risk was similar within the two patient cohorts for sensitivity (0.60 vs. 0.65; Bryan-ADRC vs. ADNI) but showed greater specificity for the Bryan-ADRC patients (0.81) than the ADNI patients (0.61). These findings reflect the different disease prevalence in the two collections resulting from approaches used to create the cohorts (Bryan-ADRC cognitively normal subjects followed prospectively, ADNI normal, MCI, and AD subjects at time of entry). The diagnostic standards are also different for the two cohorts: the Bryan-ADRC cohort is single site with a clinical diagnosis; ADNI is multi-site; and AOO was either estimated at screening for AD patients or obtained from cognitively normal individuals who converted to MCI/AD during the study; follow-up intervals are also different. Prevalence in specific populations is an important consideration for utilization of the biomarker. Measures such as PPV, NPV, odds ratio, and NRIs vary accordingly with the unique properties of the cohort, including prevalence. Measures including sensitivity, specificity, and AUC correspond only to the ability of the biomarker or algorithm to correctly identify individuals who will convert from normal cognition to MCI/AD. We have included the former measures to assess more fully their performance in cohorts that have similarity to planned clinical trials for prevention of AD. Individuals recruited for clinical trials from memory clinics will likely have considerably higher prevalence for MCI than the general public.

ROC and comparative analysis of AUC showed that the combinations of APOE genotype, TOMM40'523 genotype, and age outperformed age in sensitivity and specificity for prediction of phenoconversion. For the Bryan-ADRC

Table 3
Logistic regression modeling of age, APOE genotype, and TOMM40'523 genotype for 5-year conversion from cognitively normal to MCI or Alzheimer’s disease

| Model terms | Bryan-ADRC | | ADNI | |
|-------------|------------|-----------------|-----------------|
| Age         | 0.65 (0.03) | 0.56 (0.02) | | |
| APOE genotype | 0.68 (0.03) | 0.69 (0.02) | | |
| 523 genotype | 0.65 (0.03) | 0.67 (0.02) | | |
| Age + APOE | 0.81 (0.03) | 0.72 (0.02) | | |
| Age + 523 | 0.79 (0.02) | 0.70 (0.02) | | |
| Age + APOE+523 | 0.82 (0.02) | 0.72 (0.02) | | |
| Best model | 0.82 (0.02) | 0.72 (0.02) | | |
| Age | 0.65 (0.03) | 0.56 (0.02) | | |
| APOE genotype | 0.68 (0.03) | 0.69 (0.02) | | |
| 523 genotype | 0.65 (0.03) | 0.67 (0.02) | | |
| Age + APOE | 0.81 (0.03) | 0.72 (0.02) | | |
| Age + 523 | 0.79 (0.02) | 0.70 (0.02) | | |
| Age + APOE+523 | 0.82 (0.02) | 0.72 (0.02) | | |
| Best model | 0.82 (0.02) | 0.72 (0.02) | | |

Abbreviations: APOE, apolipoprotein E; AUC, area under the curve; SE, standard error; MCI, mild cognitive impairment; Bryan-ADRC, Joseph and Kathleen Bryan, Alzheimer’s Disease Research Center; BIC, Bayesian information criterion; ADNI, Alzheimer’s Disease Neuroimaging Initiative; TOMM40, translocase of outer mitochondrial membrane 40 homolog.

Fig. 5. Age sensitivity of GBRA illustrates the relationship between sensitivity and specificity with age. Baseline corresponds to the algorithm as defined. All age-dependent thresholds were increased or decreased by 2 and 4 years and the resulting sensitivity and specificity were plotted. Abbreviations: GBRA, genetics-based biomarker risk algorithm; Bryan-ADRC, Joseph and Kathleen Bryan, Alzheimer’s Disease Research Center; ADNI, Alzheimer’s Disease Neuroimaging Initiative.
cohort, the combination of age and the two genotypes outperformed age and either genotype (APOE or TOMM40) in terms of sensitivity and specificity. For the replication ADNI cohort, the performance of age combined with either or both genotypes was equivalent. For both cohorts, the combination of age and APOE genotype provided slightly better performance than age and TOMM40 genotype, although the difference was not statistically significant. It was surprising that the AUC for the GBRA did not show a statistically significant improvement for the ADNI cohort when compared with the combination of APOE genotype and age considering the statistical significance of the age interaction terms. Testing in additional replication cohorts from longitudinal observational studies with more precise estimation of AOO of MCI/AD will be needed to more clearly define the performance of the GBRA.

The relationship between the genetic (APOE and TOMM40/523) and age components of the GBRA and overall sensitivity and specificity is consistent for both the Bryan-ADRC and ADNI cohorts; improved test performance is observed for slightly younger versus older ages. This age sensitivity has also been demonstrated in studies examining ADNI cohort patients with respect to interactions between APOE and changes in both neurocognitive and neuroimaging end points [28]. In addition, in a study that compared the effects of TOMM40/523 and APOE genotype on preclinical longitudinal memory decline, age sensitivity was also observed. There was a significant TOMM40/523 effect before age 60 characterized by flattened test-retest improvement for individuals carrying the TOMM50-0523 VL/VL genotype, but no significant APOE effect, and a significant APOE effect after age 60 [15]. It is notable that as age thresholds are increased, the GBRA becomes less sensitive and more specific. This is potentially a desirable quality for clinical trial enrichment where specificity is particularly important because screening is relatively low cost, but enrollment of false-positives in a trial cohort is expensive. It is unclear why sensitivity decreases with increasing the age.

![Graph](image)

**Fig. 6.** Comparative performance of the GBRA, CSF (combination of amyloid β and tau), and fMRI biomarkers. Abbreviations: GBRA, genetics-based biomarker risk algorithm; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; HC, healthy control; fMRI, functional magnetic resonance imaging.

### Table 4

| GBRA risk group | CSF levels (ADNI) | Imaging (ADNI) | MMSE** |
|-----------------|------------------|----------------|--------|
|                 | Aβ42**         | p-tau**        | SUVR*  |
|                 |                 |                | ADNI   |
| High            | 157.15 ± 3.78   | 36.70 ± 1.28   | 1.90 ± 0.06 |
| Low             | 195.59 ± 4.67   | 29.17 ± 1.58   | 1.52 ± 0.07 |

| GBRA risk group | CDR global | CDR sum of boxes |
|-----------------|------------|------------------|
|                 | ADNI**     | ADRC**           |
| High            | 0.45 ± 0.02| 0.52 ± 0.04      |
| Low             | 0.31 ± 0.02| 0.17 ± 0.03      |

| GBRA risk group | ADNI**     | ADRC**           |
|-----------------|------------|------------------|
| High            | 1.95 ± 0.09| 2.41 ± 0.22      |
| Low             | 1.33 ± 0.11| 0.73 ± 0.20      |

Abbreviations: GBRA, genetics-based biomarker risk algorithm; CSF, cerebrospinal fluid; ADNI, Alzheimer’s Disease Neuroimaging Initiative; MMSE, mini-mental state examination; Aβ42, amyloid-beta; p-tau, phosphorylated tau; CDR, clinical dementia rating; ADRC, Alzheimer’s Disease Research Center; SUVR, standardized uptake value ratio.

NOTE. *P < .001; **P < .0001.
There are conflicting reports on whether the TOMM40*523 variant has an APOE-independent effect on AD risk and AOO [6,12,16,29–31]. The primary study that supports the use of both APOE and *523 for LOAD risk prediction is a single site (Duke; Bryan-ADRC), prospective study with a clinical diagnosis [6,12,32]. APOE-independent effects of TOMM40*523 are also reported in studies of episodic memory [15,33,34] and brain imaging changes associated with early AD expression [35]. The contradictory studies involve meta-analysis of large cohorts that were collected for Genome Wide Association Studies (GWAS); these studies noted that there was no statistically significant independent effect of TOMM40*523 genotype above APOE genotype on either AD risk or AD AOO [30,31]. There is high linkage disequilibrium and in turn, statistical correlation between the APOE and TOMM40*523 variants which limits assessment of independent effects by statistical conditional analysis [36]. There were also substantial differences in the studies considered for the genetic association analyses. Some of the component studies were prospective, where incident cases were captured in the course of the study. Other studies were cross-sectional, or had a cross-sectional component, and would have contributed cases with retrospectively reported AOO data where recall and survivor biases are likely to undermine the accuracy of the data. Roses et al. [16] provides a comprehensive summary of factors to be considered for replication studies of AOO of disease.

A limitation of the present study is the modest size and length of time participants have been observed in the ADNI and Bryan-ADRC cohorts, and, therefore, appropriate caution must be exercised when interpreting the existent data. Although the performance characteristics in the Bryan-ADRC and ADNI of the GBRA are encouraging, long-term longitudinal studies in additional larger, diverse neurodegenerative disease cohorts are needed. As additional testing data are accrued, refinement of the GBRA will continue, potentially leading to improved prognostic performance and broad applicability to both clinical drug development and epidemiologic studies in the future.

A decline in neurocognition is the hallmark of AD and neocognitive testing has routinely been demonstrated as the most informative measure of future cognitive decline [37]. Using the biomarker and neocognitive testing data from the ADNI cohort, Gomar et al. [38] found that cognitive measures were the best predictors of conversion from MCI to AD. It is encouraging that the GBRA performs consistently with respect to the decline of neocognitive measures. This suggests that these biomarkers represent features that are integral to or an important part of the cognitive decline characteristic of AD.

Utilization of any biomarker is dependent not only on its performance characteristics (NPV and PPV) but also by the intended application, often referred to as “fit for purpose.” In addition to the test performance, features such as availability of specialized reagents, instruments and/or qualified personnel, willingness of subjects to be tested, invasiveness, cost/reimbursement, and consideration of the clinical utility in the practice of medicine contribute to the use of any given test. Any use of a biomarker to enrich a clinical trial in normal subjects at risk for conversion to disease symptoms needs to consider how that test would be used in the practice of medicine in a global environment. The GBRA relies on genomic DNA using robust, widely available inexpensive testing of an analyte that is unaffected by environmental, disease state, or other conditions. A two-stage process is also possible, where the GBRA would be used to initially screen subjects for a prevention trial using an inexpensive blood test followed by CSF and/or imaging biomarkers for further screening of individuals likely to convert in the time period of the trial. Although the GBRA was developed as a binary predictive algorithm for a delay-of-disease-onset clinical trial of cognitively normal subjects to high- and low-risk groups, the algorithm could also be adapted to a continuous scale based on likelihood of conversion within a prespecified time frame.

Combinations of biomarkers could conceivably be used to further refine selection of subjects for clinical trials, balancing cost and invasiveness of the assay with the improvement in accuracy of conversion prediction in a prespecified time frame. There is strong precedent for the combination of APOE, CSF, imaging biomarkers, and neocognitive measures to improve predictive accuracy [39], and recent work using the BIOCARD cohort [40,41] with 20 years of longitudinal follow-up has provided promising results [42]. Specifically, for prediction of the onset of MCI in a 5-year time frame, the combination of six measures provided the best performance: Two memory and thinking tests (the digit symbol and paired associates immediate recall tests), levels of CSF, Aβ, and p-tau, and two MRI brain scans—one to assess the thickness of the right entorhinal cortex and another to measure the volume of the hippocampus. Accuracy of prediction was reported as AUC = 0.89, sensitivity = 0.85, and specificity = 0.70 [42].

The data summarized previously suggest that age-dependent risk of developing MCI due to AD can be stratified informatively according to TOMM40*523 and APOE genotypes. This information has contributed to the design of a pharmacogenetically enriched, double-blind, delay-of-disease-onset clinical trial of cognitively normal subjects...
aged 65–83 years, inclusive, classified as having high or low risk for development of cognitive symptoms over the course of a 5-year study (TOMORROW trial; ClinicalTrials.gov identifier = NCT01931566 [16]). In this study, high-risk subjects are randomized to active therapy or placebo; low-risk subjects are randomized to placebo only. Stratification for risk of developing MCI due to AD during the study before randomization is accomplished with the GBRA at the beginning of the study (when neuropsychological testing verifies normal cognition). The genetic GBRA will be qualified for use as a prognostic biomarker at the end of the phase 3 trial when the performance characteristics and ROC curves can be calculated from the trial data which will provide a large (n > 3000), prospectively sampled cohort. Once qualified, the biomarker can be used as a companion pharmacogenetics test for a therapeutic to delay the onset of AD.

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RESEARCH IN CONTEXT

1. Systematic review: We used standard sources of biomedical literature, recent reports from regulatory agencies including the FDA and European Medical Agency, information learned at scientific meetings, and through contacts with colleagues to search, review, and evaluate accumulated knowledge regarding biomarkers for prediction of the risk of Alzheimer’s disease (AD) in the context of research, clinical trials, and clinical practice.

2. Interpretation: The genetic biomarker risk algorithm (GBRA) described in this study is a prognostic biomarker algorithm that demonstrates equivalence to other AD biomarkers in widespread use such as cerebrospinal fluid levels of amyloid-beta 42 and phosphorylated tau and structural imaging (functional magnetic resonance imaging) of amyloid-beta burden. The GBRA is also a good prognostic indicator of neurocognitive scores; an indication of its utility for enrolling subjects of high-risk for cognitive decline in clinical outcome trials of AD therapies and its relevance to fundamental changes associated with AD. The results from this study support the concept that age-dependent risk of developing mild cognitive impairment (MCI) due to AD can be stratified informatively according to translocation of outer mitochondrial membrane 40 homolog ‘523 and apolipoprotein E genotypes.

3. Future directions: The GBRA described in this study is a component of a pharmacogenetically enriched, double-blind, delay-of-disease onset (MCI due to AD), TOMORROW trial; ClinicalTrials.gov identifier = NCT01931566. Stratification for risk of developing MCI due to AD during the study before randomization is accomplished with the GBRA. The GBRA will be qualified for use as a prognostic biomarker at the end of the phase 3 trial when the positive predictive value and negative predictive value of the GBRA can be calculated from the trial data which will provide a large (n = 5800), prospectively sampled cohort. Once qualified, the biomarker can be used as a companion PGx test for a therapeutic to delay the onset of AD. Future studies will examine the performance characteristics of the GBRA for non-Caucasian ethnicities and will continue to evaluate the utility of this biomarker in the context of research and clinical applications for AD prevention and treatment.
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