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Biomarkers of Alzheimer's Disease Among Mexican Americans

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Abstract

Background—Mexican Americans are the fastest aging segment of the U.S. population yet little scientific literature exists regarding the Alzheimer disease (AD) among this segment of the population. The extant literature suggests that biomarkers of AD will vary according to race/ethnicity though no prior work has explicitly studied this possibility. The aim of this study was to create a serum-based biomarker profile of AD among Mexican American.

Methods—Data were analyzed from 363 Mexican American participants (49 AD and 314 normal controls) enrolled in the Texas Alzheimer's Research & Care Consortium (TARCC). Non-fasting serum samples were analyzed using a luminex-based multi-plex platform. A biomarker profile was generated using random forest analyses.

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Results—The biomarker profile of AD among Mexican Americans was different from prior work from non-Hispanic populations with regards to the variable importance plots. In fact, many of the top markers were related to metabolic factors (e.g. FABP, GLP-1, CD40, pancreatic polypeptide, insulin-like-growth factor, and insulin). The biomarker profile was a significant classifier of AD status yielding an area under the receiver operating characteristic curve (AUC), sensitivity (SN) and specificity (SP) of 0.77, 0.92 and 0.64, respectively. Combining biomarkers with clinical variables yielded a better balance of SN and SP.

Conclusion—The biomarker profile for AD among Mexican American cases is significantly different from that previously identified among non-Hispanic cases from many large-scale studies. This is the first study to explicitly examine and provide support for blood-based biomarkers of AD among Mexican Americans. Areas for future research are highlighted.

Keywords

Biomarkers; Mexican American; Alzheimer's disease; Neuropsychology

Introduction

AD is the most common neurodegenerative dementia with over 5.4 million Americans suffering from the disease; every 71 seconds an American develops AD [1]. AD is the 5th leading cause of death for those over 65[1]. As illustrated in Figure 1, the percentage of Hispanics 65 and above will triple by the year 2050[2] (see Figure 1) and rates of AD are expected to grow six-fold among Hispanics[3]. Approximately 65% of Hispanics in the U.S. are Mexican American[4], making them the fastest aging segment of the population. However, there remains a dearth of scientific literature examining AD among Mexican Americans. Research from our group and others suggests that a significant health disparity exists as Mexican Americans may (1) be at increased risk for AD[3], (2) are diagnosed at a more advanced stage of disease progression[5], (3) develop AD at younger ages[5, 6], (4) have a lower frequency of the ApoEε4 allele (the strongest genetic risk for AD among non-Hispanic whites)[6, 7], and (5) suffer from a disproportionate burden of modifiable risk factors for AD (e.g. diabetes)[6, 8]. Therefore, there remains a significant need for research on AD among this underserved ethnic group[3, 5, 6, 9].

In recent years, there has been a surge in research aimed at the identification of blood-based biomarker of AD that cover a wide range of biological systems[10–15]. Investigators have sought to identify diagnostic biomarkers [16–23], biomarkers of future AD disease risk[24–29], as well as rate of disease progression [30–33]; however, no such work has been demonstrated clinic-ready to date[34]. Despite significant advancements, little work has investigated the impact of race or ethnicity on AD biomarkers, whether blood-based or other modalities. This is also the case for many broader-based AD research questions [35]. Given the rapidly increasing Mexican American population, this underserved community will face a disproportionate burden of AD in the near future and additional research is needed specific to this ethnic group.

Despite the lack of research attention, empirical evidence supports the notion that biological markers associated with AD may be different for Hispanics and Mexican Americans. Jun and colleagues[36] recently conducted a meta-analysis of genome-wide allelic association study (GWAS) data from several large-scale cohorts that included over 500 Caribbean Hispanics AD cases. In this study, ApoEε4 genotype was significantly associated with AD status among all ethnic groups, including Hispanics. While CLU (SNP rs11136000), CR1 (SNP rs3818361) and PICALM (SNP rs3851179) were associated with AD status among non-Hispanic whites, these genetic markers were not associated with AD status in any other

ethnic group (African American, Israeli-Arab, or Hispanic). Given that ApoEε4 frequency is less common among Mexican Americans [6, 7], additional work is needed to identify other genetic contributors to this disease among this ethnic group. Bertoli Avella et al[37] identified a novel presenilin 1 mutation (L174 M) that was associated with early onset AD among a large Cuban family. Therefore, current work suggests variability in susceptibility genes of AD by race/ethnicity, which suggests that proteins related to the disease will also likely vary.

To date, a scant literature exists on the link between blood-based biomarkers and cognition among Hispanics, or Mexican Americans in particular. A series of studies have examined the relation between blood-based markers and cognition as well as dementia status among the Sacramento Area Latino Study on Aging (SALSA) cohort[38–40]. In this cohort, folate levels were shown to be related to cognitive functioning and dementia diagnosis[38] while homocysteine levels were shown to be associated with neuropsychological functioning[40] as well as risk for incident dementia[39]. However, none of this work has specifically examined blood-based biomarkers of AD among Mexican Americans. We recently analyzed serum C-reactive protein (CRP) data from 471 Mexican Americans (AD n=55, MCI n=91, control n=325) and 595 non-Hispanic whites (AD n=229, MCI n=134, NC n=232) enrolled into the Texas Alzheimer's Research and Care Consortium (TARCC). In the non-Hispanic portion of this cohort, CRP levels were significantly decreased among AD cases as compared to controls, which is consistent with other work[13, 30, 41]; however, CRP levels were not related to such outcomes among Mexican Americans [42]. The notion of CRP being differentially related to clinical outcomes by ethnicity is consistent with prior work. Veeranna and colleagues[43] conducted archival analysis of data from 6,067 participants (2362 Caucasian, 1601 African Americans, 1353 Hispanics, 751 Chinese) from the Multi-Ethnic Study of Atherosclerosis (MESA) to examine the link between baseline blood-based biomarkers, including CRP and incidence of cardiovascular disease (CVD). CRP levels were higher among Hispanics as compared to Caucasians; however, CRP level was a significant predictor of incident CVD among Caucasians, but not among Hispanics. Therefore, additional work is needed to examine biomarkers of AD among Mexican Americans, as well as other ethnic minorities. The current study sought to (1) generate a serum-based biomarker profile of AD among Mexican Americans, (2) compare the biomarker profile to our prior work with non-Hispanics and (3) evaluate the utility of this preliminary blood-based biomarker profile in discriminating between Mexican American AD cases and normal controls.

Methods

Participants

Data were analyzed from 363 Mexican American participants (AD n=49, control n=314) of the Texas Alzheimer's Research & Care Consortium (TARCC). Each participant underwent a standardized evaluation at the respective TARCC site, which included an interview (e.g. demographics, family dementia history, NSAID, Vitamin E & anti-dementia medication history, medical history), neuropsychological testing and non-fasting blood draw. Global cognition was assessed via the Mini Mental State Examination (MMSE)[44] and disease severity rated according to the Clinical Dementia Rating scale [45] sum of boxes scores (CDR SB)[46, 47]. An informant interview was conducted for each research participant to obtain information regarding his/her activities of daily living (basic and instrumental). All information was reviewed by consensus committee who diagnosed participants as of NINCDS-ADRDA Probable Alzheimer's disease[48] or cognitively normal control (NC) if they performed within normal limits on psychometric assessment[49]. Interviews and assessments were conducted in Spanish or English depending on the participant's preference. Demographic characteristics of the sample are presented in Table 1.

Assays

Non-fasting samples were collected with 10mL serum-separating (tiger-top) vacutainers tubes at the time of interview. Samples were allowed to clot at room temperature for 30 minutes in a vertical position before being centrifuged at 1300 × g for 10 minutes. Next, 1mL aliquots were pipetted into polypropylene cryovial tubes and placed in −20° C (non-frost free) or −80° C freezers until shipment to TARCC Biobank. All samples from the current project were shipped in to Myriad Rules Based Medicine (Myriad RBM) in a single batch for assay on the luminex-based HumanMAP 1.0 platform. Over 100 proteins were quantified utilizing fluorescent microspheres with protein-specific antibodies. Information regarding the least detectable dose (LDD), inter-run coefficient of variation, dynamic range, overall spiked standard recovery, and cross-reactivity with other humanMAP analytes can be obtained from Myriad Rules Based Medicine. Buffy coats were extracted from EDTA plasma collection tubes (purple top) for DNA extraction using Puregene® isolation kits. ApoEε4 genotyped was conducted using standard PCR methods[50].

Statistical Analyses

Analyses were performed using SPSS 19 (IBM) and R (V 2.10) statistical software[51]. Chi square and t-tests were used to compare case versus controls for categorical variables (ApoEε4 allele frequency, gender) and continuous variables (age and education), respectively. The biomarker data was log transformed and then standardized for each analyte and the sample randomly spilt into a training (AD n=25, control n=157) and test set (AD n=24, control n=157). Using only the training set, a random forest (RF) biomarker profile was generated using R package *randomForest* (V 4.5)[52], with all software default settings. The RF Gini plot was examined to compare the top 30 markers from Mexican Americans to the top 30 from our prior work with non-Hispanic whites[22]. The RF biomarker risk score was then applied to the test sample to determine the ability of the biomarker profile in discriminating AD cases from normal controls. The ROC (receiver operation characteristic) curves were analyzed using R package AUC (area under the curve) was calculated using R package *DiagnosisMed* (V 0.2.2.2). ROC curves reflect sensitivity and 1-specificity for all possible scores on an item with AUC reflective of the overall discriminability based on the ROC figure.

Results

Demographic characteristics of the sample can be found in Table 1. When compared to normal controls, the AD cases were significantly older (t=0.53, p<0.001), achieved significantly fewer years of education (t=2.65, p=0.008), obtained significantly lower MMSE scores (t=20.52, p<0.001), and were rated higher on the CDR SB scores (t=31.3, p<0.001).

First, the biomarker profile was generated using Random Forest within the training set. As can be seen, among the top 30 markers 22 were overexpressed among AD cases while 8 were under-expressed. The overall biomarker profile was then compared to the profile of the top 30 markers previously identified among non-Hispanics in our prior work [22]. When examining the RF Gini plot, the top 30 serum-based biomarkers related to AD status among Mexican Americans was significantly different from the top markers found in our prior work with biomarkers among non-Hispanics whites (see Figure 2 and Table 2). The fold change for the markers can be found in Table 2.

Next, the biomarker risk score was applied to the test set to determine the classification accuracy of the profile. A cut off of 0.126 on the RF risk score yielded an AUC of 0.77 (95% CI = 0.69–0.85) with a sensitivity of 0.92 (95% CI= 0.74–0.98) and specificity of 0.64

(95% CI= 0.57–0.71)(see Figure 3 and Table 3). The biomarker risk score yielded a superior sensitivity to clinical data, but inferior specificity. Clinical variables alone (age, gender, education and ApoEε4 genotype) yielded an AUC of 0.88 (95% CI= 0.81–0.95), sensitivity of 0.83 (95% CI=0.64–0.93) and specificity of 0.78 (95% CI= 0.71–0.84). Of note, only age ($p<0.001$) and ApoEε4 genotype ($p=0.003$) were significant contributors among the demographic factors. When the biomarker risk score and demographic factors were combined into a single predictive algorithm, as done in our prior work [21–23], the AUC of 0.88 (95% CI= 0.81–0.96) remained unchanged from the clinical data alone. However, the combined algorithm yielded a better overall balance of sensitivity and specific with an increase in sensitivity to 0.92 (95% CI=0.74–0.98) with a small decrease in specificity (0.73, 95% CI=0.65–79).

Discussion

There are several important findings from the current study. First, our prior methods can be utilized to generate a biomarker profile of AD among Mexican Americans [21–23]. However, when using the same biomarker multiplex platform containing over 100 proteins, the biomarker profile of AD among Mexican Americans was substantially different from that previously observed among non-Hispanic whites. Second, a significant classifier of AD can be generated for Mexican Americans though the blood-based algorithm is less accurate than what has been identified among non-Hispanic whites leaving room for improvement. This latter finding is likely due to the small sample size in the current study and the current algorithm is preliminary in nature. To our knowledge, this is the first project to explicitly search for blood-based biomarkers of AD among Mexican Americans.

The differential biomarker profile of AD among Mexican Americans is not surprising. First, as pointed out previously, the genetic markers associated with AD appear to be different when non-Hispanics and Hispanics are compared [36, 37] and ApoEε4 is less common among Mexican Americans [6, 7]. Additionally, Crean and colleagues [53] recently demonstrated that ApoEε4 prevalence rates fluctuate globally, which supports this notion. If the genetics of the disease vary by ethnicity, the proteins associated with the disease must also vary. Second, many of the protein markers that have been previously linked to AD have also been linked to other health conditions, such as diabetes, obesity, insulin resistance and the metabolic syndrome, which are more common among Mexican Americans [6, 54–57]. Given this increased prevalence of these metabolic conditions among Mexican Americans, it is possible that a metabolic phenotype of AD is more important in this population than among non-Hispanic whites. In fact, in the current study, the number one marker in the risk score was fatty acid binding protein (FABP). Fatty acid binding proteins are small intracellular cytoplasmic proteins involved in the binding, transport and metabolism of long-chain free fatty acids [58, 59] that have been implicated in diabetes, obesity, insulin resistance and the metabolic syndrome. Interestingly, genetic analyses of FABPs have also shown ethnic variation and many haplotypes were only related to clinical outcomes among non-Hispanic whites [59]. For example, the Ala54Thr FABP2 polymorphism was specifically associated with type 2 diabetes (T2DM) among Hispanic Americans [58], and a new SNP of FABP5 (rs454550) specifically associated with T2DM among non-Hispanic whites and African Americans [58]. Other markers in the biomarker profile of AD among Mexican Americans have also been associated with metabolic conditions including CD40 [60], glucagon-like peptide 1[61, 62], pancreatic polypeptide [63], β2-microglobulin [64], insulin-like-growth factor [65], peptide YY [66], insulin, and TSH [67]. Therefore, the current findings may point towards a metabolic endophenotype of AD among Mexican Americans and this possibility requires further attention. The notion of metabolic endophenotypes related to neurological and neuropsychiatric conditions has been proposed for both Autism [68] and schizophrenia [69], which should provide the groundwork for such

investigations in AD. The biomarker profile also included markers related to inflammation, infection, protease inhibition, iron- and oxygen binding, and oxidative stress.

Several of the markers in the current Mexican American AD profile overlap with those identified using the same biomarker assay platform among different cohorts, all of which were primarily non-Hispanic white. In the current sample, five of the top 30 markers (pancreatic polypeptide CRP, tenacin C, MIP1α, and prostatic acid phosphatase) overlapped with our prior serum-only based work among non-Hispanics from TARCC with two of these (pancreatic polypeptide and CRP) overlapping with recent work examining biomarkers of AD across Penn and Wash U cohorts [41]. An additional four markers (fatty acid binding protein, β2-microglobulin, IL18 and VCAM1) overlapped with our prior work examining AD biomarkers across serum and plasma examining data from TARCC and the Alzheimer's Disease Neuroimaging Initiative (ADNI) [23]. Several markers overlapped with those recently published by the Australian Imaging Biomarker and Lifestyle study (AIBL) [70], including insulin-like-growth factor binding protein 2, pancreatic polypeptide, MIP1α, carcinoembryonic antigen, tumor necrosis factor receptorlike2, angiopoietin 2, VCAM1, superoxide dismutase, α1-antitrypsin, β2-microglobulin, and CD40. Together these findings are supportive of overlap across AD blood-based biomarkers among Mexican Americans and non-Hispanics, but also point to (1) different markers and (2) different overall profiles (i.e. relative importance) of the biomarkers themselves (see Table 2).

There are several weaknesses to the current study. First, the sample size of AD cases is small and the accuracy of the biomarker algorithm, therefore, should be viewed as preliminary. Our group is currently recruiting larger numbers of Mexican Americans with and without AD with future studies planned to cross-validate and expand the current findings (including into Mexican Americans with Mild Cognitive Impairment). Second, while the biomarker platform contained a large number of markers (over 100), there are likely additional markers not investigated that will be of importance among Mexican Americans. Third, detailed information regarding metabolic conditions is not available among the TARCC database and therefore, additional analyses into a metabolic endophenotype of AD among Mexican Americans will be conducted utilizing a different cohort currently being established. Despite these limitations, the current project is the first to explicitly examine blood-based biomarkers of AD among Mexican Americans. The results suggest that the biomarker profile of AD among this underserved ethnic group is different from that of non-Hispanic whites. This hypothesis is supported by other work related to the genetics and biomarker analyses of AD and associated risk factors (e.g. diabetes, metabolic syndrome, obesity, insulin resistance) among Mexican Americans. These findings point towards the need for additional work aimed at understanding AD among Mexican Americans.

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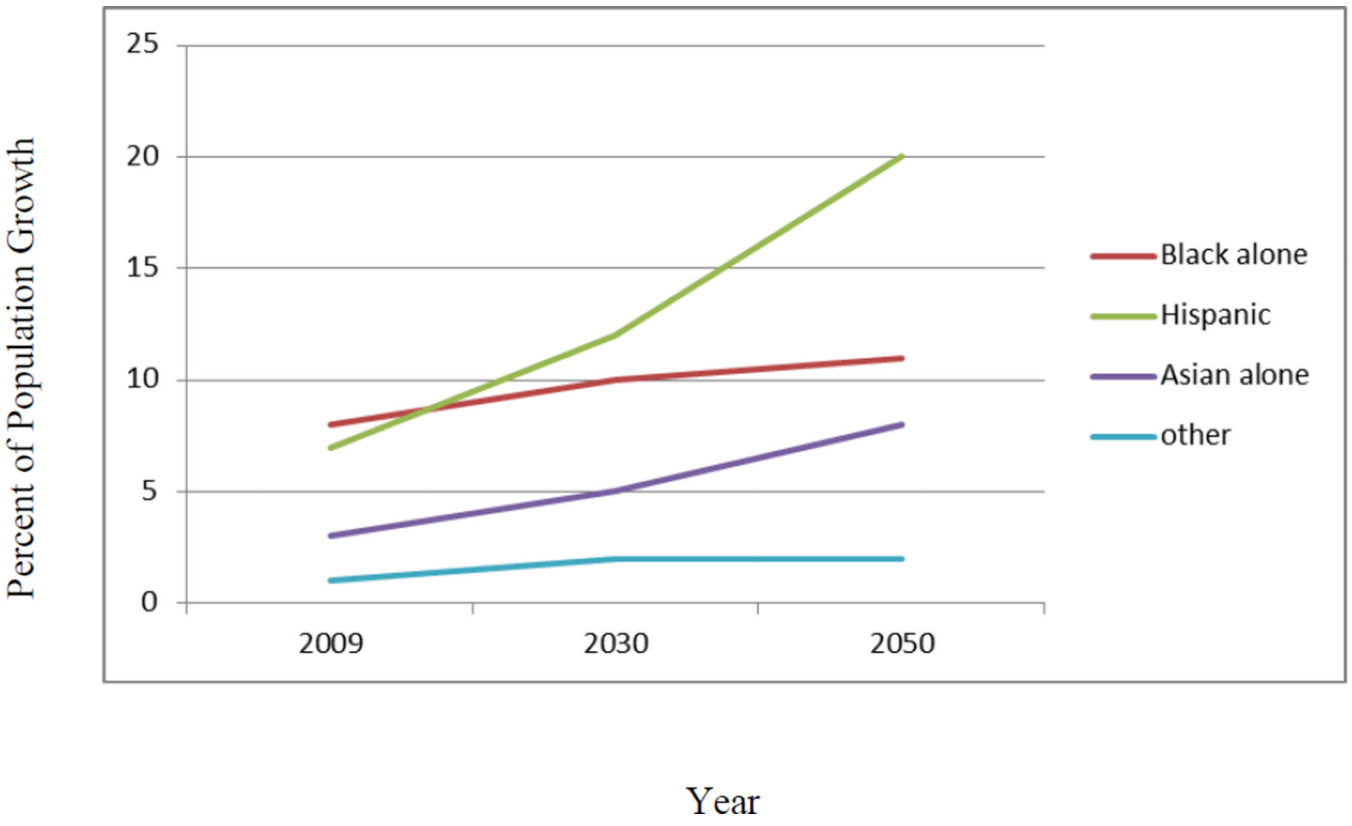

Figure 1.
Projected Growth of Minority Elder Population Age 65 and Above in the U.S.

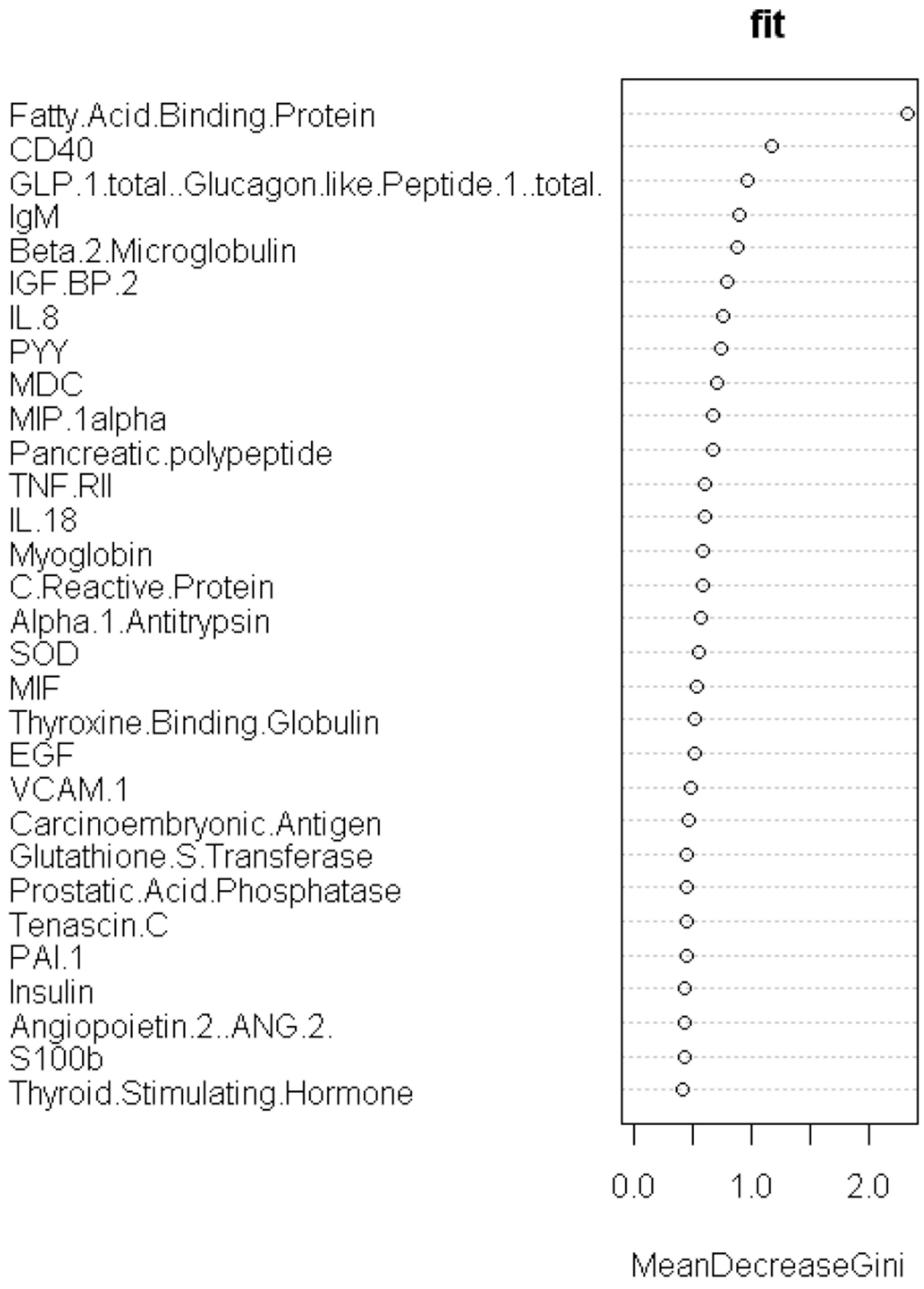

Figure 2.
Gini Plot of top 30 serum biomarkers of AD among Mexican Americans

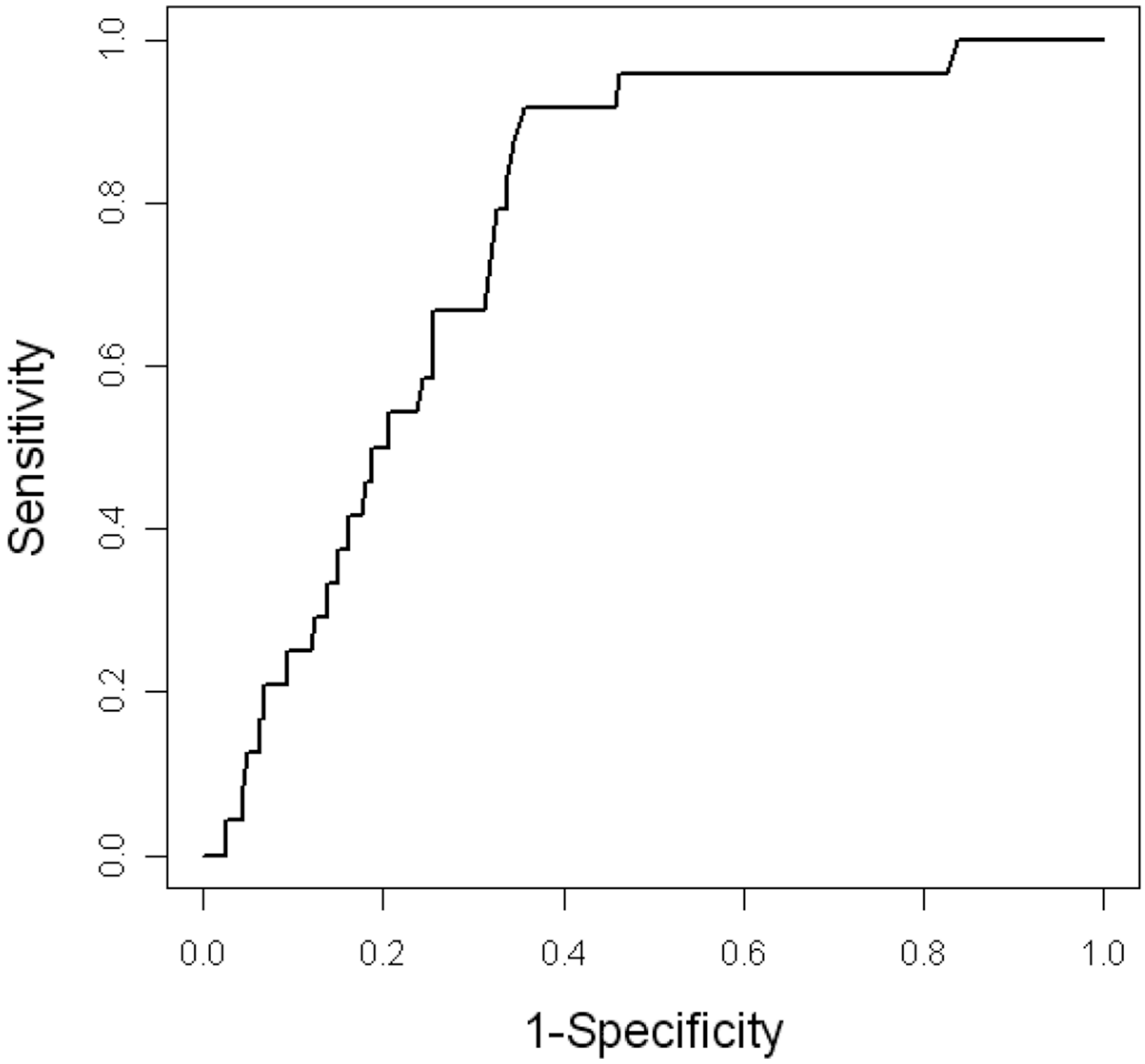

Figure 3.
ROC figure for biomarker profile of AD among Mexican Americans

Table 1

Demographic Characteristics of Mexican Americans in the Cohort

	AD (N=49)	Control (N=314)	P-value
Gender (male)	41%	37%	0.37
Age	76.5 (7.6) 61–94	65.0 (8.0) 50–87	<0.001
Education	9.2(5.5) 0–20	11.2(4.7) 0–20	0.008
MMSE	19.5(4.9) 9–28	28.3(2.3) 19–30	<0.001
CDR SB	5.4(3.0) 2–13	0.02(0.18) 0–3	<0.001
ApoEε4 carrier (yes/no)	47% *	13% *	0.002

NOTE:

* ApoEε4 genotype only available on 17 of the AD cases and 147 of the controls

Table 2

Top 30 markers associated with AD among Mexican Americans as compared to non-Hispanic whites

	Top 30 biomarkers of AD among Mexican Americans	Fold Change of Mexican American biomarkers	Top 30 biomarkers previously identified among non-Hispanic whites[22]	Fold Change among non-Hispanic whites
1.	Fatty Acid Binding Protein✓	1.70	Thrombopoietin	2.18
2.	CD40*	1.29	MIP1α	0.70
3.	Glucagon like peptide 1	1.21	Eotaxin 3	1.26
4.	IgM	0.67	TNFα	0.74
5.	Beta 2 Microglobulin✓*	1.34	Creatine kinase	0.80
6.	IGF BP2*	1.61	Tenascin C	1.60
7.	IL8	1.09	FAS	1.03
8.	Peptide YY	1.69	Fibrinogen	0.87
9.	Macrophage-derived chemokine	1.09	IL 10	0.76
10.	MIP1α✓*	1.31	IL 7	1.02
11.	Pancreatic polypeptide ✓*	1.50	Cancer antigen 19 9	1.09
12.	TNF RII*	1.30	Prostatic acid phosphatase	0.78
13.	IL18✓	0 98	Apolipoprotein CIII	1.12
14.	Myoglobin	1.34	Fas ligand	0.85
15.	CRP✓	0.75	CRP	0.86
16.	α1-antitrypsin*	1.16	Pancreatic polypeptide	1.33
17.	Super oxide dismutase*	1.24	TIMP 1	0.99
18.	Migration inhibitory factor	1.50	Angiopoietin 2	0.95
19.	Thyroxine binding globulin	0.95	Stem cell factor	0.74
20.	EGF	1.06	IL 5	0.92
21.	VCAM1✓	1.17	Lipoprotein a	1.07
22.	Carcinoembryonic antigen*	1.34	α2-macroglobulin	2.45
23.	Glutathione S transferase	0.69	ACE CD143	1.01
24.	Prostatic acid phosphatase✓	1.04	MCP 1	0.85
25.	Tenascin C✓	1.23	Ferritin	0.97
26.	PAI1	0.95	PARC	1.12
27.	Insulin	0.79	Cancer Antigen 125	1.11
28.	Angiopoietin2*	1.21	Von Willebrand Factor	1.29
29.	S100b	1.57	Carcinoembryonic Antigen	1.10
30.	TSH	0.99	MIF	1.11

NOTE:

✓denotes overlap with serum biomarkers identified among non-Hispanics by O'Bryant and colleagues[23];

*denotes overlap with plasma biomarkers identified among non-Hispanics by Doecke[69]

Table 3

Classification Accuracy of Biomarker Profile among Mexican Americans

	AUC	Sensitivity	Specificity
Biomarker alone	0 77 [0 69 – 0 85]	0 92 [0 74 – 0 98]	0 64 [0 57 – 0 71]
Clinical alone	0 88 [0 81 – 0 95]	0 83 [0 64 – 0 93]	0 78 [0 71 – 0 84]
Biomarker + Clinical	0 88 [0 81 – 0 96]	0 92 [0 74 – 0 98]	0 73 [0 65 – 0 79]

NOTE: AUC = area under the receiver operating characteristic, sensitivity = proportion of true positives correctly identified by the test; specificity = proportion of true negatives that are correctly identified by the test