RESEARCH ARTICLE

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Genetic characterization of the ALFA study: Uncovering genetic profiles in the Alzheimer's *continuum*

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Abstract

INTRODUCTION: In 2013, the ALzheimer's and FAmilies (ALFA) project was established to investigate pathophysiological changes in preclinical Alzheimer's disease (AD), and to foster research on early detection and preventive interventions.

Natalia Vilor-Tejedor and Patricia Genius contributed equally to this study.

METHODS: We conducted a comprehensive genetic characterization of ALFA participants with respect to neurodegenerative/cerebrovascular diseases, AD biomarkers, brain endophenotypes, risk factors and aging biomarkers. We placed particular emphasis on amyloid/tau status and assessed gender differences. Multiple polygenic risk scores were computed to capture different aspects of genetic predisposition. We additionally compared AD risk in ALFA to that across the full disease spectrum from the Alzheimer's Disease Neuroimaging Initiative (ADNI).

RESULTS: Results show that the ALFA project has been successful at establishing a cohort of cognitively unimpaired individuals at high genetic predisposition of AD.

DISCUSSION: It is, therefore, well-suited to study early pathophysiological changes in the preclinical AD *continuum*.

KEYWORDS
AD *continuum*, ALFA study, Alzheimer's disease, neurogenetics, neurological diseases, prevention

Highlights

- Prevalence of $\varepsilon 4$ carriers in ALzheimer and FAmilies (ALFA) is higher than in the general European population
- The ALFA study is highly enriched in Alzheimer's disease (AD) genetic risk factors beyond *APOE*
- AD genetic profiles in ALFA are similar to clinical groups along the *continuum*
- ALFA has succeeded in establishing a cohort of cognitively unimpaired individuals at high genetic AD risk
- ALFA is well suited to study pathogenic events/early pathophysiological changes in AD

1 | BACKGROUND

In 2013, the ALzheimer's and FAmilies (ALFA) project was launched by the Barcelonaβeta Brain Research Center with the aim of enhancing our understanding of the pathogenesis and pathophysiology of Alzheimer's disease (AD) at its early preclinical stages.[1] The ALFA project consists of the ALFA parent cohort (NCT01835717), and the nested ALFA+ cohort study (NCT02485730). The ALFA parent cohort is composed of 2743 cognitively unimpaired participants aged between 45 and 74 years at the time of recruitment who underwent cognitive testing, clinical history, magnetic resonance imaging (MRI), environmental and lifestyle questionnaires, and blood sampling. A significant feature of ALFA is that it is a genetically enriched cohort of cognitively unimpaired individuals, as ∼50% of them are adult children of patients with AD dementia diagnosed before the age of 75 years. The recruitment strategy of the ALFA parent cohort has resulted in a population that is enriched in genetic risk factors for AD, the most notable of which is the *apolipoprotein E* (*APOE*)-$\varepsilon 4$ allele. A subset of the ALFA parent cohort participants were invited to participate in a nested longitudinal long-term study, which is referred to as ALFA+. This study involved more detailed phenotyping, including fluid (blood and cerebrospinal fluid [CSF]) and positron emission tomography (PET). ALFA+ participants were selected according to their age, sex, and *APOE-$\varepsilon 4$* carriership to cover the full range of the AD risk spectrum.

In a pilot study (NCT02198586), ∼575 ALFA parent cohort participants underwent MRI to investigate the effects of *APOE* variants on different cerebral phenotypes. As a result of this project, we found that $\varepsilon 4$-homozygous displayed an age-related increase in radial but not axial diffusivity, consistent with a reduced myelin sheath in several white matter regions.[2,3] When assessing gray matter volumes in *APOE-$\varepsilon 4$* homozygous, we found reductions in brain regions known to undergo atrophy in symptomatic AD stages[4] and dose-dependent protective effects of the *APOE-$\varepsilon 2$* allele.[5] We also showed, as suggested in previous studies, that *APOE-$\varepsilon 4$* reversed the association between cognitive performance and brain morphology, similar to aging, suggesting that this risk allele leads to an accelerated biological phenotype of brain aging.[6] Furthermore, $\varepsilon 4$-homozygous displayed reduced connectivity between networks in areas typically susceptible to amyloid deposition in the early AD *continuum*, and altered effects of amyloid on brain structure.[7,8] Additional results indicated that cognitively

unimpaired *APOE-ε4* homozygous were at significantly higher risk of having pathological levels of white matter hyperintensities (WHM) than heterozygotes,[9] and observed a protective effect of the *ε2 allele* on global WMH.[10] We also found a higher prevalence of cerebral microbleeds in *APOE-ε4* carriers,[11] although no association was found between *APOE-ε4* and the enlargement of perivascular spaces.[12]

These findings have encouraged us to extend the genetic characterization of the ALFA parent cohort beyond *APOE* to deepen our understanding of the biological mechanisms associated with genetic predisposition to AD at preclinical stages, which may aid in the implementation of prevention programs. In this article, we present the rationale, methods, and genetic characterization of participants of the ALFA parent cohort. In addition, we assessed AD genetic risk prediction across the spectrum of the disease by including data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) project.[13]

2 | METHODS

2.1 | Design

On the September 17, 2012, a press conference was held where the main aims of the ALFA project were presented along with detailed inclusion and exclusion criteria.[1] Briefly, participants had to be cognitively unimpaired Spanish and/or Catalan-speaking individuals aged 45–74 who agreed to undergo clinical interviews and questionnaires associated with dementia risk factors, cognitive tests, a blood sample extraction for DNA analysis, and MRI. A total of 2743 individuals were recruited in the ALFA parent cohort, with 86.3% reporting a diagnosis of AD in at least one of their parents. When considering a more strict family history encoding, 47.4% of the ALFA study participants had at least one of their parents that had been diagnosed with AD before the age of 75 years. In total, 2686 participants were genotyped, as 57 individuals were excluded since blood extraction could not be performed or sufficient DNA could not be obtained to perform the genotyping. The genetic data processing procedure is detailed in Figure S1. Summary information on project participants (Figure 1), DNA extraction,

RESEARCH IN CONTEXT

1. **Systematic review**: The ALFA (ALzheimer and FAmilies) project prospectively follows cognitively unimpaired late-/middle-aged participants, most of whom are adult offspring of Alzheimer's disease (AD) patients, with the aim of investigating pathophysiological events that occur during the preclinical stages of AD and to test preventive interventions. We aimed here to genetically characterize ALFA participants and to describe AD risk prediction across the disease spectrum.

2. **Interpretation**: The ALFA study population is highly enriched for AD genetic risk factors beyond apolipoprotein E (*APOE*), defined by individuals in the early stages of AD. ALFA study participants show heterogeneous genetic profiles similar to clinical groups along the AD *continuum*. Results support that the ALFA parent cohort and, even more, ALFA+ are highly enriched for genetic risk factors for AD. This made them suitable for investigating the early pathophysiological alterations in AD.

3. **Future directions**: Integration with other -omic data modalities and risk factors, as well as collaborating with global initiatives will be crucial to discover underlying disease mechanisms.

genotyping, and data availability is described in Supplementary methods. A subset of ALFA parent cohort (*N* = 380), referred to as ALFA+, also underwent lumbar puncture to determine biomarkers in CSF, enhanced MRI, more detailed cognitive testing, blood sampling for biomarker determination, 18F-fluoro-2-deoxyglucose PET, amyloid, and tau PET imaging. Participants were classified into A/T groups defined by their biomarker profile according to the A/T framework described in Ref.[14] Briefly, CSF amyloid-β 42 (Aβ42) levels of ≤1098 pg/ml were designated as A+, and phosphorylated tau (pTau) levels of ≥24 pg/ml were designated as T+.[15]

alfa study
ALZHEIMER FAMILIES
Blood samples from ALFA study participants (N=2686)

Genome-wide genotyping, Quality Control (QC) and genetic Imputation

Sample after genetic QC

- **2527** cognitively unimpaired participants (range age: 45-75 yo)
- **High proportion** of AD patients' offspring.
- **High proportion** of *APOE*-ε4 carriers.

- Clinical history, cognition (**N=2527**)
- Environmental & Lifestyle factors (**N=2425**)
- Telomere length (**N=1600**)
- Magnetic Resonance Imaging (**N=1426**)
- PET scans (**N=286**)
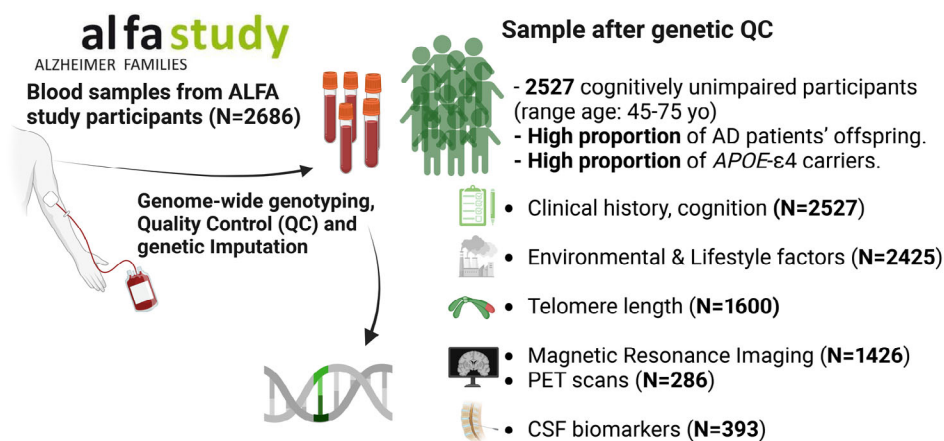- CSF biomarkers (**N=393**)

FIGURE 1 Summarized information of the characteristics of project participants.

2.2 | Genetic quality control and imputation

Quality control (QC) of genetic data was performed with the PLINK software. Samples with a call rate of less than 98%, mismatched genetically determined sex (from X-chromosome heterogeneity) versus that coming from demographic data, or excess of heterozygosity (four standard deviations from the mean) estimated by an F statistic, were excluded from the analyses. Individuals at high genetic relatedness (at the level of cousins or closer relatives), sharing proportionally more than 18.5% of alleles (IBD > 0.185), were also excluded. After completion of sample level QC, genetic variants with low minor allele frequency (MAF < 1%), a Hardy-Weinberg equilibrium ($p < 10^{-6}$), and missingness rates >5% were also excluded. Population stratification was checked by clustering the samples using principal component analysis. After the QC procedure, a total of 2527 cognitively unimpaired individuals from the ALFA parent cohort were genetically characterized.

Imputation of genetic variants was carried out using the Michigan Imputation Server with the *Haplotype Reference Consortium* Panel (HRC r1.1 2016)[16,17] following default parameters and established guidelines. Mismatched and monomorphic genetic variants were removed during pre-imputation filtering. Genetic variants with invalid alleles were switched and flipped in the genome-wide association studies (GWAS) sets (Human Reference Panel: GRCh37 hg19 b37 humanG1Kv37). Phasing was performed using the EAGLE software v.2.4, and imputation results were rechecked using standard Michigan imputation server QC parameters (imputation quality >0.2 and MAF >1%).

2.3 | Statistical analysis

Differences in demographic characteristics were assessed according to *APOE-ε4* carriership, AT groups and sex, using chi-square tests for categorical variables and parametric (*t*-test, ANOVA) and non-parametric tests (Wilcox test or Kruskal-Wallis) for continuous normally and non-normally distributed variables, as appropriate. Pairwise comparison *p*-values were also provided, adjusted for false discovery rate correction.

Polygenic risk scores (PRS) were computed using PRSice version 2[18] to assess polygenicity. PRSice computes PRS by summing all SNP alleles carried by participants, weighting them by the SNP allele effect size estimated in a previous GWAS, and normalizing the score by the total number of SNPs included. PRS were calculated in representative genetic variants per linkage disequilibrium block (LD) (clumped variants), using a cutoff for LD of $r^2 > 0.1$ in a 250-kb window. For PRS calculation of AD and AD CSF biomarkers, the *APOE* region was additionally excluded (chr19:45,409,011-45,412,650; GRCh37/hg19). Results were displayed at a restrictive threshold, 5×10^{-6}. A total of 40 PRS were calculated based on recently published GWAS, distributed in 6 main categories of diseases and conditions: AD biomarkers,[19] neurodegenerative diseases,[20–22] cerebrovascular diseases,[23–24] brain endophenotypes of AD,[25–27] AD risk factors,[28–35] and aging

biomarkers.[36–39] Further details on the PRS can be found in Table S1. In addition, individuals were classified into three groups based on the distribution of PRS for AD, Aβ42, and pTau: low genetic predisposition (PRS ≤ percentile 10), intermediate genetic predisposition (percentile 10 < PRS ≤ percentile 90), and high genetic predisposition (PRS > percentile 90). Additionally, significant differences in the median values (Wilcoxon test) and variances (Levene test) of the PRS were evaluated between *APOE-ε4* carriers, AT groups in the ALFA+ cohort, and sex. Finally, the AD predisposition in ALFA was compared (pairwise median test) to that across the full disease spectrum in the ADNI database by including controls (CN) (N = 530), amyloid positive individuals with mild cognitive impairment (MCI) (N = 598), and AD dementia patients (N = 205). ALFA+ and ADNI participants were further stratified according to amyloid status (ALFA+: A+ N = 134, A- N = 246; ADNI: CN A+ N = 96, MCI A+ N = 264, AD A+ N = 132).

3 | RESULTS

3.1 | Demographic characteristics and *APOE* genotype distribution

A characteristic feature of the ALFA study is the high prevalence of *APOE-ε4* carriers compared with the general European population (35.6% vs. 14%; $p < 0.001$),[40] and the high prevalence of homozygotes in the ALFA+ subsample (Table 1, Figure 2). Significant differences in demographic characteristics were found between *APOE-ε4* non-carriers (N = 1624; 64.39%) and carriers (N = 898; 35.61%). Non-carriers were on average older than *ε4*-homozygous individuals ($p = 0.024$). The percentage of women in the non-carrier group was higher than in the *APOE-ε4* heterozygous one ($p = 0.038$). We also observed a higher percentage of individuals with a family history of AD in *APOE-ε4* heterozygous compared to non-carriers ($p < 0.001$) (Figure 2A, Table S2). In ALFA+, a total of 8% (N = 31) of the individuals were *APOE-ε4* homozygous. Moreover, among *APOE-ε4* carriers, 8.2% of individuals were A+T+ (vs. 7.5% among non-carriers), 41.3% were A+T- (vs. 10.9% among non-carriers), and 50.5% were A-T- (vs. 81.6% among non-carriers) (Figure 2B, Table S3, and S4). Notably, there was a higher percentage of A+ individuals among *APOE-ε4* carriers compared with non-carriers (49.5% vs. 18.4%; $p < 0.001$). Similar to the overall sample, the proportion of women was higher in the non-carrier group compared to the *APOE-ε4* heterozygous one ($p = 0.029$). No significant differences were found in the distribution of individuals according to family history of AD.

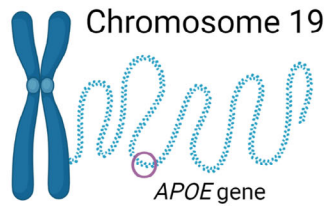### 3.2 | Polygenic characterization of ALFA participants

PRS of AD (PRS-AD) showed the highest variability in the sample (IQR = 0.25) as well as frontotemporal dementia (FTD) subtypes (IQR > 0.035), sleep risk factors and biological aging phenotypes

TABLE 1 Demographic characteristics and APOE genotypes distribution in the ALFA parent cohort as well as in the ALFA+ subsample

	ALFA parent cohort (N = 2527)	N	ALFA+ subsample (N = 380)	N
Age (years, median)	56.00 [51.00;62.00]	2527	57.00 [53.75;61.00]	380
Sex (*n*, %):		2527		380
Men	932 (36.88%)		149 (39.21%)	
Women	1595 (63.12%)		231 (60.79%)	
Education (years, median)	12.00 [10.00;17.00]	2527	12.00 [11.00;17.00]	380
Mini Mental State Exam (0-30, median)	29.00 [28.00;30.00]	803	30.00 [29.00;30.00]	68
APOE-ε4 load (*n*,%)		2522		380
Non-carriers	1624 (64.39%)		174 (45.79%)	
One ε4 allele	810 (32.12%)		175 (46.05%)	
Two ε4 alleles	88 (3.49%)		31 (8.16%)	
APOE-ε4 status (*n*, %)		2522		380
Non-carriers	1624 (64.39%)		174 (45.79%)	
Carriers	898 (35.61%)		206 (54.21%)	
Family history (FH) of AD (*n*,%)		2527		380
No	294 (11.63%)		28 (7.37%)	
Yes	2233 (88.37%)		352 (92.63%)	
Parental family history of AD (*n*,%)		2527		380
Both	97 (3.84%)		19 (5.00%)	
Father	679 (26.87%)		107 (28.16%)	
Mother	1457 (57.66%)		226 (59.47%)	
No Family History of AD	294 (11.63%)		28 (7.37%)	
Family history of AD (*n*,%)		2527		380
age of onset < 75	1436 (56.83%)		232 (61.05%)	
age of onset > = 75	866 (34.27%)		120 (31.58%)	
at least one < 75	72 (2.85%)		16 (4.21%)	
both > = 75	45 (1.78%)		7 (1.84%)	
No FH	108 (4.27%)		5 (1.32%)	
Amyloid status (*n*, %)				380
Aβ42/Aβ40-			246 (64.74%)	
Aβ42/Aβ40+			134 (35.26%)	
AT classification (*n*, %)				380
A-T-			246 (64.74%)	
A+T-			104 (27.37%)	
A+T+			30 (7.89%)	
CSF Aβ142 (pg/ml, median)			1160.00 [852.80; 1563.50]	376
CSF pTau181 (pg/ml, median)			14.18 [11.09; 18.59]	377
Centiloids (whole cerebellum) (median)			−1.32 [−6.52; 5.30]	324

Note: A total of 5 individuals presented NA values in APOE-ε4 characterization. A total of 13 individuals with A-T+ profile were excluded from the ALFA+ subsample. Available CSF biomarkers data after outliers removal. A total of 56 individuals presented NA values in Centiloids. Amyloid positivity is defined as Aβ42/Aβ40 < 0.071.

Abbreviations: ALFA, ALzheimer's and FAmilies; *APOE, apolipoprotein E*; CSF, cerebrospinal fluid.
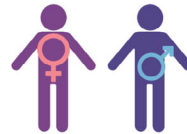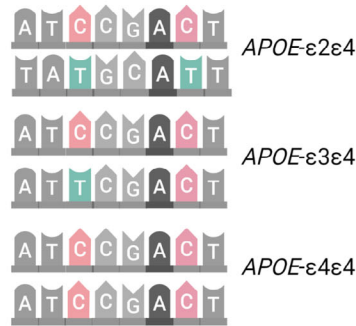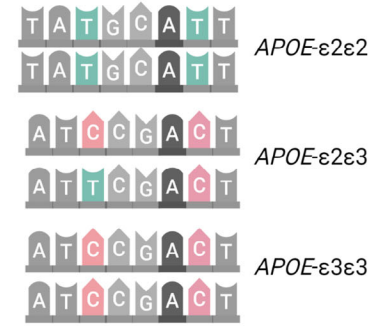
FIGURE 2 Distribution of *APOE-ε4* carriers in the ALFA and ALFA+ samples. ALFA, ALzheimer's and FAmilies; *APOE*, apolipoprotein E.

(IQR > 0.04), whereas Parkinson's disease (IQR = $3 \cdot 10^{-5}$), cerebral infarction (IQR = $8 \cdot 10^{-5}$), and intracerebral hemorrhage (IQR = $2 \cdot 10^{-5}$) were the main conditions in which subjects had more homogeneous scores (IQR ~ 0) (Figure 3, Table S5).

We observed that *ε4* homozygous had higher scores for AD and FTD, AD biomarkers, and some brain endophenotypes, risk factors and aging conditions compared with heterozygotes and non-carriers (Figure S2). Moreover, we observed that A+T- individuals had higher scores for PRS-AD ($p = 0.006$) as well as lower median PRS of CSF Aβ42 levels than A-T- ($p \sim 0$). In addition, A+T- showed higher genetic predisposition to WMH than A-T- ($p = 0.006$). Similarly, A+T- showed a higher

median for the risk score of microbleeds and longer sleep duration than A-T- ($p < 0.018$) (Figure S3, Table S6). Finally, only significant differences were found between sex for genetic predisposition to social isolation and sleep duration (Figure S4, Table S7).

3.3 | Characteristics of individuals with AD genetic predisposition

We found significant differences in the percentage of *ε4* carriers among individuals classified at high (81%), intermediate (33.3%), and low
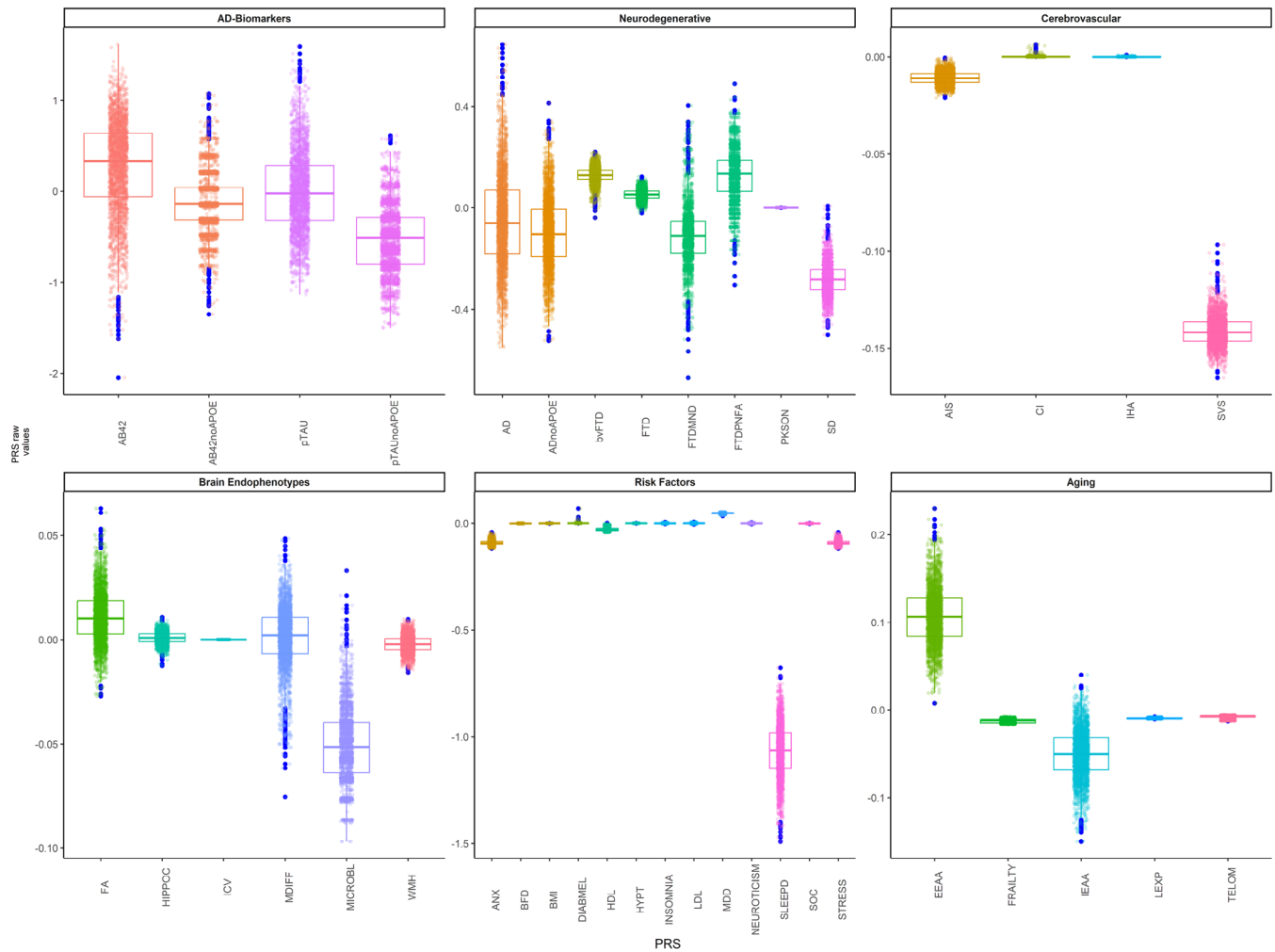
FIGURE 3 Distribution of genetic scores in the ALFA parent cohort. ALFA, ALzheimer's and FAmilies.

(7.94%) genetic predisposition to AD ($p < 0.001$) (Table S8). When the *APOE* region was excluded from the PRS-AD, the proportion of $\varepsilon 4$ carriers and non-carriers was balanced. When classifying individuals based on both the PRS-AD and the PRS-AD$_{noAPOE}$, the proportion of individuals with a positive family history of AD was higher in the group with high genetic predisposition to AD compared to the rest of groups. We did not find any differences in the proportion of women and men across groups. In ALFA+, we also observed a higher percentage of $\varepsilon 4$ carriers among individuals at high genetic predisposition to AD (89.39%) compared to individuals at intermediate genetic predisposition (50.53%, $p < 0.001$) and low (12.90%, $p < 0.001$) genetic predisposition. In the high genetic group, 21.2% of the individuals were $\varepsilon 4$-homozygous (Table S9). The proportion of A+T- and A+T+ was also significantly higher in the group at higher genetic predisposition of AD compared to the intermediate and low groups ($p < 0.05$). When removing the *APOE* region from the genetic score computation these significant differences were no longer observed. When we classified individuals according to their genetic predisposition to higher CSF-Aβ42 levels, we found that the proportion of *APOE-$\varepsilon 4$* carriers

and, specifically, homozygotes, was significantly higher in the genetically predisposed group to present abnormal levels of CSF-Aβ42 (low genetic predisposition to higher CSF-Aβ42 levels) compared to the other groups ($p < 0.001$). However, when we removed the *APOE* region from the PRS-Aβ42, we did not observe significant differences in the percentage of *APOE-$\varepsilon 4$* carriers among groups (Table S10). Similarly, we found a higher proportion of *APOE-$\varepsilon 4$* carriers, and specifically, homozygotes, in the group of individuals at higher genetic predisposition to display higher CSF-pTau levels (at risk group) ($p < 0.001$). However, we did not find these differences when removing the effect of the genetic variants in the *APOE* region (Table S11). The above results were also assessed for the subgroup of individuals with available CSF biomarkers from the ALFA+ study. Significant differences were also found in the distribution of *APOE-$\varepsilon 4$* carriers among genetic groups based on their genetic predisposition to CSF Aβ42 levels (Table S12). As in the whole sample, higher percentages of both *APOE-$\varepsilon 4$* heterozygous and homozygous were found in the group at lower genetic predisposition to higher CSF Aβ42 levels compared with the high and intermediate groups ($p < 0.001$). Moreover, in this group we found a
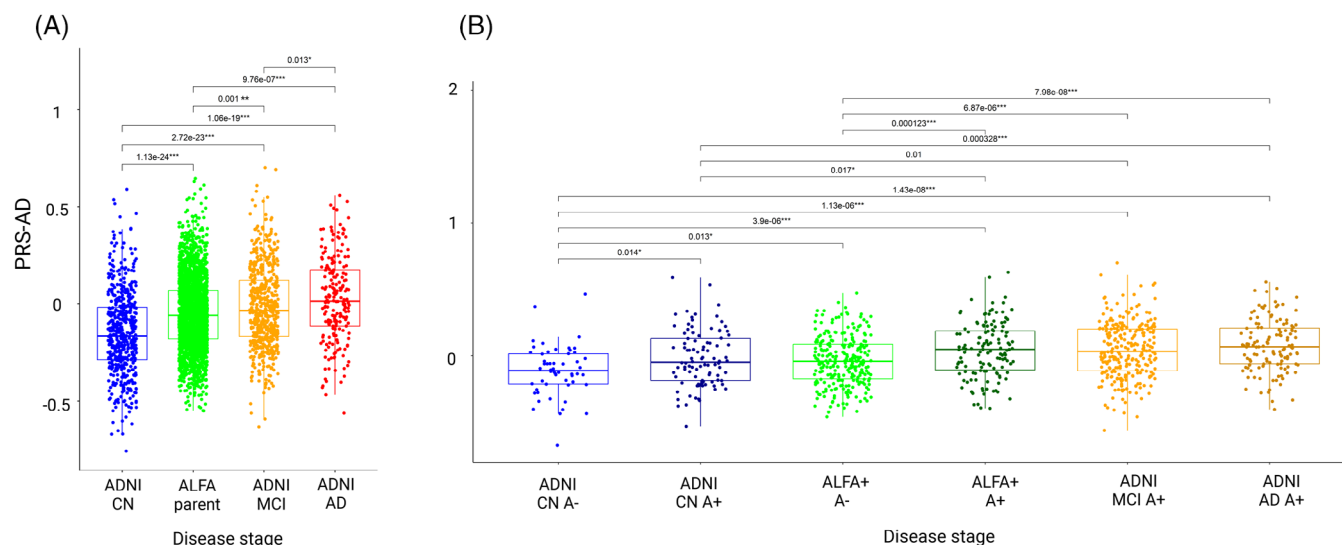
FIGURE 4 Distribution of genetic scores of AD along the AD *continuum*. (A) Subgroups from ALFA and ADNI within the AD *continuum*. (B) Subgroups within the AD *continuum* stratifying ALFA participants by amyloid status. Pairwise comparisons are assessed to compare the median PRS-AD (Wilcoxon test) among groups. Significant results at nominal *p*-value are displayed (*p*-value < 0.05*; *p*-value < 0.01**, *p*-value < 0.001***). AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; ALFA, ALzheimer's and FAmilies; PRS-AD, polygenic risk scores of AD.

higher percentage of A+ individuals compared with the intermediate (*p* = 0.008) and high (*p* = 0.008) genetic groups. In the low genetic group, we found a higher percentage of A+T+ individuals than in the higher group (*p* = 0.012) but lower than in the intermediate group (*p* = 0.005). These differences were not observed when removing the *APOE* region. Significant differences were also observed between genetic groups based on their genetic predisposition to CSF pTau levels (Table S13). As in the whole sample, higher percentages of both *APOE-ε4* carriers and homozygous were found in the group showing high genetic predisposition to higher CSF pTau levels compared with the low and intermediate groups (*p* < 0.001). Moreover, in the high group, we found a higher percentage of A+ individuals as well as a higher percentage of A+T+ individuals compared with the low and intermediate genetic groups (pairwise comparisons *p* < 0.05). Non-significant differences were observed after removing the *APOE* region.

3.4 | AD genetic risk prediction in the whole disease spectrum

The median value of the PRS-AD increased along the AD *continuum* (Figure 4). In ADNI (Table S14), significant differences in the median score of PRS-AD were observed between CN and MCI (*p* < 0.001) and between CN and AD (*p* < 0.001). Significant differences were also found between MCI and AD subjects (*p* < 0.05). When participants from the ALFA parent cohort were included in the spectrum, we found that the median value of the PRS-AD in ALFA participants was higher than in CN from ADNI (*p* < 0.001) although was significantly lower than in MCI (*p* < 0.01) and AD (*p* < 0.001) (Figure 4A). When ADNI and ALFA+ individuals were stratified by Aβ status, we found that the

genetic predisposition to AD was higher in ALFA+ A+ than in ALFA+ A- (*p* < 0.001) (Figure 4B). Moreover, the median PRS-AD in ALFA+ A+ individuals was higher compared both to CN A- (*p* < 0.001) and CN A+ (*p* < 0.05). Although there were no significant differences between them, ALFA+ A+ individuals showed a higher median value of the PRS-AD than MCI A+. Non-significant differences were found between ALFA+ A+ and AD individuals (Table S15). Comparisons stratifying by other risk factors were assessed (Figure 5). We found significant differences among *APOE-ε4* groups for all disease-stage groups (Figure 5A). The higher the number of *ε4* alleles, the higher the median value of the PRS-AD. Neither in ALFA A- nor in A+ significant differences were found in the median PRS-AD between *ε4* allele heterozygous and homozygous. Nonetheless, in both groups, both *ε4* allele heterozygous and homozygous displayed higher values for the median PRS-AD than non-carriers (*p* < 0.001). Differences were also assessed stratifying by A/T profiles (Figure 5B). In the ALFA+ sample, A+T- individuals showed higher median PRS-AD than A-T- (*p* < 0.001). In ADNI, CN that were A+T+ displayed higher median values than A-T- (*p* < 0.01). In MCIs, significant differences were found between all pairwise comparisons (*p* < 0.05), while in AD patients, non-significant differences were found in the median value of the PRS-AD among AT groups. Finally, we did not observe significant sex-differences within ADNI nor in ALFA groups (Figure 5C). Moreover, when removing the *APOE* region, the median score was significantly lower (*p* < 0.05) in CN A- compared to AD A+ from ADNI (Figure S5A, Table S16). Also, we consistently observed a higher median score in ALFA+ A+, MCI A+, and AD A+ groups, specifically for less restrictive inclusion thresholds (Tables S17–S20). An extended characterization of other neurodegenerative and aging-related scores along the AD *continuum* can be found in the Supplementary Results documentation.
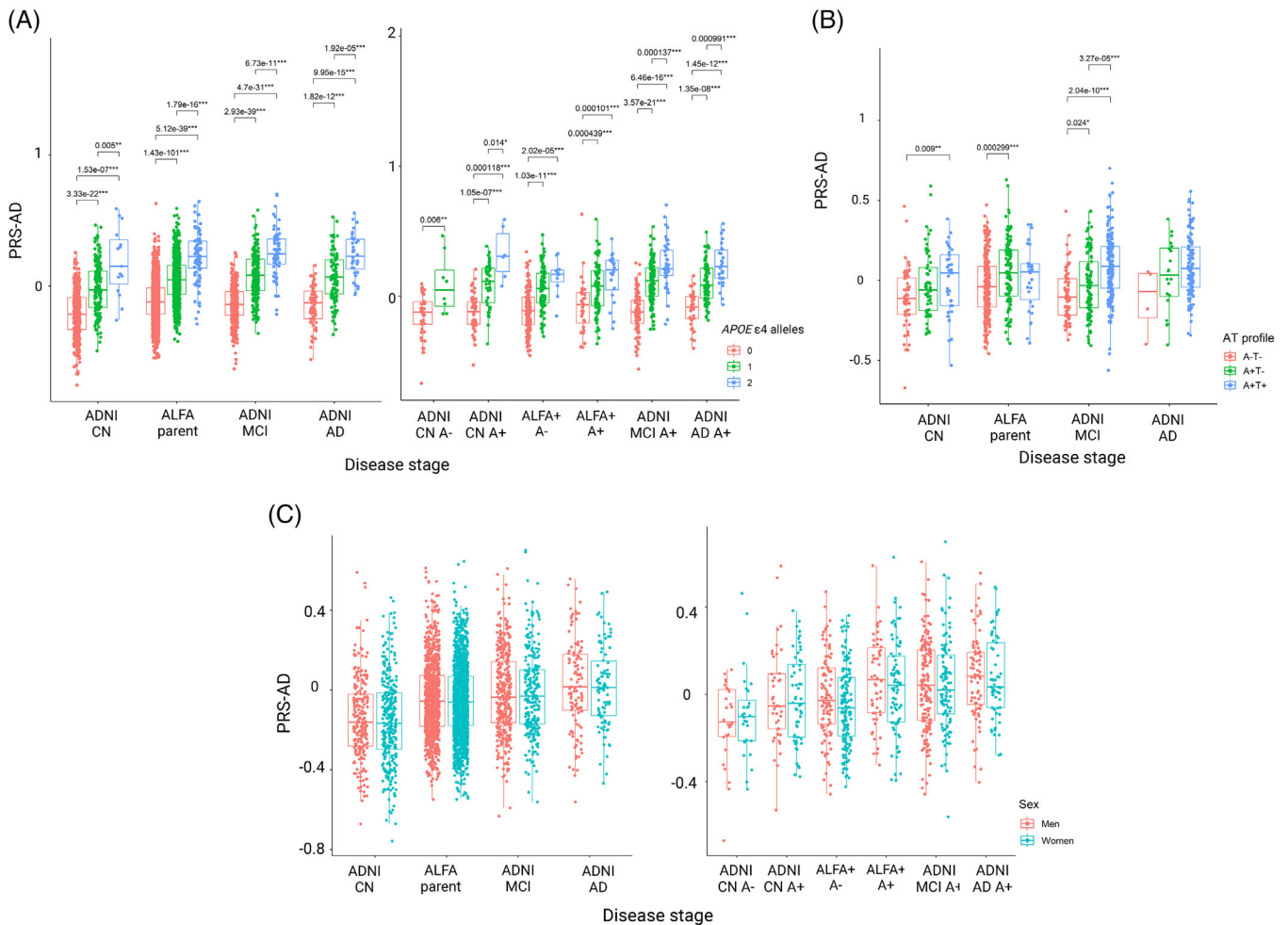
FIGURE 5 Distribution of genetic scores of AD along the AD *continuum* stratified by risk factors. (A) Distribution stratified by *APOE-ε4* carriership. (B) Distribution stratified by AT group. (C) Distribution stratified by sex. Pairwise comparisons are assessed to compare the median PRS-AD (Wilcoxon test) among groups. Significant results at nominal *p*-value are displayed (*p*-value < 0.05*; *p*-value < 0.01**, *p*-value < 0.001***). AD, Alzheimer's disease; *APOE*, apolipoprotein E; PRS-AD, polygenic risk scores of AD.

4 | DISCUSSION

The genetic characterization of ALFA parent cohort participants enabled us to investigate the genetic predisposition to neurodegenerative and other complex diseases in cognitively unimpaired individuals, many of whom were in the preclinical AD *continuum*. Our results showed a significant enrichment of genetic risk factors for AD in the ALFA parent cohort, further highlighting its suitability for studying early pathophysiological alterations in AD. Specifically, we observed a substantial genetic predisposition to AD in ALFA participants, even in the absence of the *APOE-ε4* allele. These findings suggest that other genetic variants, independent of *APOE*, may play a role in modulating the risk of AD and potentially compensate for or complement the effect of *APOE* in genetic predisposition to the disease. Our results align with previous studies,[41,42] emphasizing the importance of considering additional *APOE*-independent mechanisms when exploring genetic predisposition to AD. This illustrates how the use of PRS can reveal additional pathophysiological pathways with potential for

prevention,[43] which is relevant since the pathophysiology of AD has not yet been fully elucidated and the best biological characterization to date focuses on the determination of AD biomarkers.[44,45] However, it is important to emphasize the contribution of *APOE* to the observed genetic enrichment. *APOE* has been well-established as a major genetic risk factor for AD, primarily associated with early alterations in Aβ42 metabolism.[46] In our study, individuals at genetic predisposition to AD displayed abnormal CSF Aβ42 levels and were enriched for *APOE-ε4* homozygosity. These findings support the notion that *APOE* plays a crucial role in the early pathogenesis of AD, while also suggesting that additional genetic variants contribute to the overall genetic risk profile.

Furthermore, comparing the PRS-AD profiles of the ALFA+ participants, particularly those already on the AD *continuum* (A+), with clinical groups in ADNI, revealed that the ALFA participants exhibited a higher genetic risk profile than CN in ADNI, and similar to that of the clinical groups (MCI, AD). These results support the notion that the ALFA cohort is well-suited for detecting early pathogenic events in AD, encompassing both *APOE*-associated and independent biological

pathways. The study also confirmed that CN individuals with abnormal AD biomarkers had a higher genetic predisposition to AD, as shown by the distribution of PRS-AD against AT groups. These results suggest that PRS could be used as a proxy for risk stratification of unimpaired individuals.[47,48] However, further research is needed to establish the additional information that PRS provide in addition to biomarkers and their utility in research and clinical practice.

Moreover, individuals showing AD pathological changes (A+T-) showed higher genetic predisposition to AD, WMH, long sleep duration, and microbleed counts compared to subjects with normal levels of AD biomarkers (A-T-). We did not detect any differences in PRS variability in T+ individuals, likely due to the low sample size of this group at the moment. These findings suggest different patterns of genetic predisposition in the Alzheimer's *continuum* and/or increased susceptibility to comorbidities .[49,50] For instance, our results imply a possible common pathological mechanism linking genetic predisposition to cerebrovascular disease and sleep measurements to the development of amyloid-beta pathology at the earliest stages of the AD *continuum*. Further studies that consider scores regardless of the inclusion of the *APOE* region will additionally give insights into the biological interpretation of the results.

We observed slight differences in variability of genetic scores between sexes. Analyses using sex-specific summary statistics will help to better determine the mechanisms associated with such differences and to understand structural and functional differences in the brain, as well as cognitive and age-related processes.[51,52] In addition, previous studies suggest the influence of sex-specific variants with small effects or more complex mechanisms involving epigenetic changes, gene-environment interactions, or genetic variants within sex chromosomes that should be incorporated into future studies.[53,54]

Methodological limitations also should be taken into account, such as the subtle arbitrariness in the selection of the threshold for including genetic variants in the computation of genetic scores, alongside the threshold for categorizing individuals into genetic risk groups.

Our research aims to systematically address these challenges and significantly impact the field by collaborating with global initiatives like the UNITED (Uncovering Neurodegenerative Insights Through Ethnic Diversity) Consortium,[55] the European Prevention of Alzheimer's Dementia (EPAD) cohort,[56] and the Quantitative amyloid PET in Alzheimer's disease (AMYPAD) initiative,[57] and using publicly available genetic data from other resources.[58] This will manifest in increased statistical power and precision in determining genetic variants for score computation, as well as in a more profound understanding of the genetic mechanisms underlying AD worldwide, while also considering diverse populations.

By leveraging the ALFA cohort's substantial genetic risk profile, our research gains a unique advantage in unraveling the initial pathophysiological alterations within the preclinical AD *continuum*. Through the incorporation of multiple PRSs and focusing on distinct genetic markers, our research not only advances the comprehension of the genetic underpinnings of AD but also reveals promising additional strengths for interventions at an early stage. The complex nature of AD furthermore adds more value to the ALFA cohort, as it propels the utilization of alternative modalities, allowing us to characterize the biological pathways involved in AD processes. This multifaceted approach enhances our understanding of how molecular differences correlate with in vivo neuroimaging phenotypes.[59] Additionally, our incorporation of techniques such as Mendelian Randomization provides a means to establish causal links between modifiable risk factors and AD pathology, including its subsequent consequences,[60] ultimately influencing the potential to establish primary and secondary prevention strategies during the primary and preclinical stages.[61]

CONFLICT OF INTEREST STATEMENT

J.L.M. is currently a full-time employee of Lundbeck and has previously served as a consultant or at advisory boards, or has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, ProMIS Neurosciences. J.D.G. has received speaker's or consultant's fees from Philips Nederlands, Roche Diagnostics and Biogen and research support from GE Healthcare, Roche Diagnostics and Hoffmann-La Roche. M.S.C. has served as a consultant and at advisory boards for Roche Diagnostics International Ltd., has given lectures in symposia sponsored by Roche Diagnostics, S.L.U., Roche Farma, S.A. and Roche Sistemas de Diagnósticos, Sociedade Unipessoal, Lda. and research support from Roche Diagnostics International Ltd. G.S.B. has served as a consultant for Roche Farma, S.A. The remaining authors declare that they have no conflict of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

The study was conducted in accordance with the directives of the Spanish Law 14/2007, of July 3 , on Biomedical Research (Ley 14/2007 de Investigación Biomédica). The ALFA study protocol was approved by the Independent Ethics Committee Parc de Salut Mar Barcelona and registered at Clinicaltrials.gov (Identifier: NCT01835717). All participants accepted the study procedures by signing the study's informed consent form that had also been approved by the same Institutional Review Board.

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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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