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Deleterious Effect of Butyrylcholinesterase K-variant in donepezil treatment of mild cognitive impairment.

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Running Title: BChE as genetic marker of donepezil response

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1Abstract

2**Background:** Donepezil is an acetylcholinesterase inhibitor frequently prescribed for the treatment of
3mild cognitive impairment (MCI) though not approved by the Food and Drug Administration for this
4indication. In Alzheimer's disease, butyrylcholinesterase (BChE) activity increases with disease
5progression and may replace acetylcholinesterase function. The most frequent polymorphism of BChE is
6the K-variant, which is associated with lower acetylcholine-hydrolyzing activity. BChE-K polymorphism
7has been studied in Alzheimer's disease progression and donepezil therapy, and has led to contradictory
8results.

9**Objectives:** To determine whether BChE-K genotype predicts response to donepezil in MCI.

10**Methods:** We examined the association between BChE-K genotype and changes in cognitive function
11using the data collected during the ADCS vitamin E/donepezil clinical trial in MCI.

12**Results:** We found significant interactions between BChE-K genotype and the duration of donepezil
13treatment, with increased changes in MMSE and CDR-SB scores compared to the common allele in MCI
14subjects treated during the 3-year trial. We found faster MMSE decline and CDR-SB rise in BChE-K
15homozygous individuals treated with donepezil compared to the untreated. We observed similar
16interactions between BChE-K genotype and steeper changes in MMSE and CDR-SB scores in APOE4
17carriers treated with donepezil compared to controls.

18**Interpretation:** BChE-K polymorphisms are associated with deleterious changes in cognitive decline in
19MCI patients treated with donepezil for 3 years. This indicates that BChE-K genotyping should be
20performed to help identify subsets of subjects at risk for donepezil therapy, like those carrying APOE4.
21BChE-K and APOE4 carriers should not be prescribed off-label donepezil therapy for MCI management.

22

Keywords: donepezil; pharmacogenetics; mild cognitive impairment; butyrylcholinesterase; Alzheimer's
disease, therapeutics, clinical trial.

1Introduction

2 Mild cognitive impairment (MCI) is a transitional state between normal age-related changes in
3 cognition and dementia [1-3]. Most amnesic MCI patients display pathological features of Alzheimer's
4 disease (AD) [1, 4]. The incidence of AD dementia is about 10 to 15 percent per year among amnesic
5 MCI compared to 1 to 2 percent among cognitively normal elderly [1, 2]. This indicates that
6 approximately 80% of amnesic MCI will develop dementia within six years, for which some but not all
7 will be due to AD.

8 Donepezil is currently the most commonly prescribed medication for the treatment of cognitive
9 symptoms in MCI and AD, even though it is not approved by the Food Drug Administration for the early
10 symptomatic stages of AD (e.g. MCI). Donepezil belongs to the acetylcholinesterase inhibitors
11 pharmacological class. It primarily blocks the breakdown of acetylcholine by selectively inhibiting the
12 acetylcholinesterase (AChE) enzymes. Donepezil also inhibits butyrylcholinesterase (BChE) activity, but
13 displays a much lower affinity towards BChE compared to AChE [5]. Both BChE and AChE are involved
14 in ACh metabolism and thus are important for the cholinergic function in the brain. The majority of
15 cholinesterase activity in healthy brain is attributed to AChE, and BChE plays only a minor role. Studies
16 have reported that during AD progression as AChE activity declines BChE activity progressively
17 increases, suggesting that BChE is replacing AChE function over time [6].

18 The most common genetic variant of BChE was named the K variant (BChE-K) in honor of
19 Werner Kalow [7]. This variant results from a missense polymorphism in *BChE* gene at nucleotide 1615
20 (rs1803274; allelic frequencies of ~ 0.16) that changes codon 539 from GCA (Ala) to ACA (Thr) at the C
21 terminus of BChE [7]. As a result of this single nucleotide polymorphism, BChE-K has a reduced
22 catalytic activity, about 30% of the usual BChE [8]. The effect of BChE K-variant in AD progression has
23 been studied over the past 20 years, and lead to contradictory results [9-14]. However, a recent meta-
24 analysis conducted by Alzgene indicates that there is no association between the K-variant and the onset
25 of AD (41 studies, OR 1.05; 95% confidence interval [0.92, 1.18], I^2 62;
26 <http://www.alzgene.org/meta.asp?geneID=74>, accessed on Aug. 18 2016).

1 Studies reporting the influence of BChE-K genotype on donepezil response are limited to its use
2in AD [11, 15, 16]. In their case-only study, Scacchi *et al.* did not find a role of the BChE-K variant on the
3efficacy of treatment with donepezil in late-onset AD (LOAD) patients. Another study in AD failed to
4detect a treatment difference in BChE-K carriers when comparing the effect of BChE genotype on the
5response to donepezil or rivastigmine, another AChE inhibitor [15]. More recently, Han *et al.* conducted a
6case-only study in Korean AD subjects treated with rivastigmine and concluded that the BChE-K allele
7was a significant predictor for a poor response [16].

8 To our knowledge a pharmacogenetics study examining the association between BChE-K
9polymorphism and donepezil response has not been reported in MCI. In the largest donepezil clinical trial
10conducted on MCI patients, donepezil was shown to reduce the progression to AD during the first year of
11therapy, but not at the end of the 3-year trial [3]. Donepezil pharmacogenetics in MCI have been limited
12to a secondary analysis in this trial, which showed that the efficacy of donepezil persisted at year two in
13MCI subjects carrying the APOE4 allele, the major genetic risk factor for AD [2, 3]. The objective of our
14study was to determine whether the treatment response to donepezil is modulated by BChE K-variant
15genotype.

16Patients and methods

17**Patients.** Men and women aged 55 to 90 with MCI, defined as a primary memory impairment with
18relative sparing of other cognitive functions. The criteria for inclusion were amnesic MCI of a
19degenerative nature, impaired memory, a Logical Memory delayed-recall score approximately 1.5 to 2 SD
20below an education-adjusted norm, a Clinical Dementia Rating (CDR) of 0.5 and a score of 24 to 30 on
21the Mini-Mental State Examination (MMSE) [3]. Subjects on donepezil received an initial dose of 5 mg
22daily which was increased to 10 mg after six weeks. The control group in our analyses includes subjects
23from the placebo and the 2000 IU of vitamin E arms. The rationale for combining these 2 groups was the
24lack of observed therapeutic effect of vitamin E [3]. All study protocols were approved by each site's
25institutional review board and all study participants provided written informed consent before
26participating in the trial and biospecimen collection.

1Genotyping. All genomic DNA samples collected during the Donepezil/Vitamin E trial were extracted
2from blood and quantified using Picogreen (Invitrogen, Carlsbad, CA, USA) before being genotyped
3using the Illumina 610Quad array (Illumina Inc., San Diego, CA, USA) at Genizon Biosciences
4(Montreal, Quebec, Canada). QC procedures were performed using the genetic analysis package PLINK
5(<http://pngu.mgh.harvard.edu/~purcell/plink/>). The donepezil/Vitamin E dataset included 574 subjects
6with BChE rs1803274 genotypes and phenotypic data at baseline; 193 were among the donepezil arm and
7381 among the donepezil naïve group (i.e., placebo and vitamin E arms).

8Outcome measures. Our preplanned outcome variables were the change from baseline on the MMSE and
9Clinical Dementia Rating - sum of boxes (CDR-SB) scores [3]. As AD progresses, MMSE scores decline
10while CDR-SB scores increase. The primary variables of interest were treatment, genotype, and duration
11of treatment in months, with their interaction used to assess the interaction between genotype and
12donepezil effect on cognitive function response. A secondary end point was the time to the development
13of possible or probable AD [3].

14Statistical analysis. All available data from 6, 12, 18, 24, 30 and 36 months were used, and differences in
15cognitive test results (dMMSE and dCDR-SB) between baseline and follow-up treatment visits were
16calculated. We used linear regression models, adjusting for age, gender and APOE4, to test the influence
17of BChE polymorphism rs1803274 on changes in MMSE and CDR-SB scores at the end of the 3-year
18trial. We used PLINK software v1.07 to estimate the beta regression coefficient for rs1803274 in each
19group. To determine whether the association was a response to treatment, or due to a main effect of
20genotype on the history of disease progression, we also tested the interaction term of rs1803274 and
21donepezil treatment (treated vs. non-treated, i.e. vit. E and placebo arms) subjects on MMSE and CDR-
22SB changes. The mixed models with autoregressive plus random effects [AR(1)+RE)] covariance
23structure were selected to assess the interaction between treatment groups, BChE-K genotype and
24duration of therapy. In addition, the interactions between treatment groups and duration of therapy were
25also stratified by BChE-K genotypes at loci rs1803274. In order to estimate whether BChE-K

1polymorphism was independently associated with MMSE and CDR-SB scores changes, besides adjusting
2for APOE4 in the model, stratified analysis by APOE4 carriers was also performed.

3 The Kaplan-Meier curves were used to estimate time to progression to possible or probable AD
4[3] and the difference of progression time between treated and control groups was tested by Cox-
5proportional hazard model. A z-test (the difference in the proportions divided by the standard error of the
6difference) was used to compare estimated survival rates at various points on the Kaplan–Meier curves (at
76, 12, 18, 24, 30, and 36 months).

8Results

9 The frequency of rs1803274 K-carriers was ~ 33% in our population (31% in the controls and
1035% in the treated group; $P > 0.05$). In the control group, no association was found between the minor
11allele of rs1803274 (K-variant of BChE) and MMSE decline or CDR-SB rise at 36 months [**Figures 1 A**
12**and C**]. However, we found that BChE K-variant is significantly associated with faster cognitive decline
13measured by MMSE and CDR-SB scores changes in subjects treated with donepezil for 36 months.
14Indeed, the K-variant, compared to the common allele, is associated with a greater decline in MMSE [-7.2
15 ± 3.4 (K homozygous), -2.2 ± 0.5 (K heterozygous) and -0.9 ± 0.4 (non-K); $P = 0.0004$] [**Figure 1B**] and
16a greater rise in CDR-SB [(4.1 ± 1.9 (K homozygous), 1.3 ± 0.4 (K heterozygous) and 1.082 ± 0.3 (non-
17K); $P = 0.040$] [**Figure 1D**]. When examining the entire cohort as a whole (i.e. placebo, Vitamin E and
18donepezil), the overall main effect of BChE-K genotype on changes of MMSE or CDR-SB scores was not
19significant at the end of the trial. This indicates that the association noted above was due to the response
20to treatment, rather than a main effect of the BChE-K genotype on the natural history of the disease
21progression.

22 Examining the data further, we also found a highly significant interaction between treatment
23duration and BChE-K genotype on MMSE decline in the treated group ($P < 0.0038$) [**Figures 2A and B**].
24A similar interaction between duration of therapy and increased CDR-SB was observed ($P < 0.0017$)
25[**Figure 2C and D**]. Our findings thus indicate a faster decline in MMSE and rise in CDR-SB scores in
26BChE-K homozygous subjects when treated with donepezil compared to placebo ($P = 0.0096$ and $P =$

10.008 for dMMSE and dCDR-SB respectively) [**Figure 2 A and B**]. However, there was no difference of progression rate between the treated and control groups at the follow-up after 36 months ($P > 0.05$, data not shown).

4 After stratification by BChE-K genotypes, inter-group differences in dMMSE and dCDR-SB were found with longer duration of donepezil therapy in BChE-K homozygous subjects compared to untreated controls ($P = 0.026$ and $P = 0.014$ for dMMSE and dCDR-SB respectively).

7 Given the fact the APOE 4 allele is the highest risk factor for late AD onset, we stratified the results between individuals carrying it or not. In APOE4 carriers treated with donepezil, the interaction between treatment duration and BChE-K genotype on MMSE decline and CDR-SB rise remained significant ($P = 0.003$ and $P < 0.0001$ for dMMSE and dCDR-SB respectively) [**Figures 3B and 3D**]. Similarly, significant interactions persisted between BChE-K genotype and faster MMSE decline and CDR-SB rise ($P = 0.011$ and $P = 0.0007$ for MMSE and CDR-SB respectively) in treated APOE4 carriers compared to untreated (**Figure 3**). In contrast, there was no significant difference among APOE4 non-carriers (supplementary **Figure S1**)

15 Models with additional adjustments for baseline MMSE and CDR-SB values were performed, as well as models excluding the vitamin E arm in the control group. The results remained similar (data not shown).

18 Discussion

19 It was previously reported that donepezil was ineffective at the end of the three year ADCS trial in MCI [3]. Our secondary pharmacogenetic analysis of the same trial not only confirmed these past conclusions but also indicates that donepezil can be deleterious if given to MCI patients carrying the K-variant of BChE and particularly in APOE4 carriers. Hence, our findings lead to the recommendation for the use of genetic testing prior to initiating the off-label use of donepezil in MCI patients.

24 First, our data shows that BChE-K genotype does not influence cognitive decline in the absence of donepezil treatment at the end of the 3-year trial. Second, we found that BChE K-carriers exhibit differential responses to donepezil compared to non-carriers, with a negative impact of donepezil

1 treatment on cognitive performance (i.e. d-MMSE and d-CDR-SB) in the MCI K-carriers at the end of the
2 trial (**Figure 1**). Lastly, our trends test indicates that the interaction between BChE-K genotype and
3 donepezil response on cognitive function is significantly associated with the duration of treatment (**Figure**
4 **42**). Additional analyses stratified by BChE-K genotypes revealed that the interaction between BChE-K
5 polymorphism and a faster cognitive decline is significant in BChE-K homozygous carriers treated with
6 donepezil compared to controls. The stratification by APOE4 carriers showed that the interaction between
7 BChE-K polymorphism and donepezil therapy is significant in APOE4 carriers but not in APOE4 non-
8 carriers.

9 Our data analysis also confirmed that the association between BChE-K genotype and a steeper
10 cognitive decline is due to the response to donepezil, rather than a main effect of the BChE-K genotype
11 on the natural history of the disease progression.

12 These differential responses by BChE-K genotype suggest that natural BChE inhibition caused by
13 a missense polymorphism at loci rs1803274 is deleterious for MCI subjects who are treated with an AChE
14 inhibitor such as donepezil. Since both AChE and BChE are involved in the hydrolysis of acetylcholine in
15 the brain [17], we can speculate that the pharmacological inhibition of AChE by donepezil and the
16 concomitant BChE inhibition due to the missense polymorphism rs1803274 in K-variant carriers lead to
17 an overload of ACh [18], which in turns has a deleterious effect on cholinergic synapses and therefore on
18 the cognitive function. For example, it was shown that the inhibition of BChE in the hippocampus of
19 AChE deficient mice (AChE^{-/-}) causes a three-to-fivefold increases of AChE levels, while ACh levels are
20 not affected when AChE is fully active (e.g. in wild-type mice) [18].

21 While the APOE4 allele is associated with an increased risk for developing AD, the interaction
22 between APOE4 and BChE-K is unclear in AD progression and AChEI therapeutic response [16, 19, 20].
23 In our study, BChE-K carriers displayed a steeper cognitive decline on MMSE and CDR-SB in donepezil-
24 treated subjects carrying APOE4. Although, we could not determine whether the interaction was stronger
25 in the presence of the APOE4 allele, it is possible that the presence of APOE4 further alters BChE activity
26 among BChE-K carriers which can trigger ACh metabolism imbalance and cognitive decline [20]. Indeed,

1Darreh-Shori *et al.* showed that BChE activity is reduced in the cerebrospinal fluid (CSF) of AD
2individuals carrying both the APOE4 allele and the BChE-K variant, despite a similar concentration of the
3BChE protein compared to the K non-carriers [20]. They also reported that MMSE scores were lower in
4subjects with low CSF BChE activity in APOE4 carriers [5].

5 Our case-control study has several limitations. Although this dataset represents the largest and
6longest pharmacogenetic dataset of BChE-K in MCI, it is nevertheless limited by the size of the BChE
7homozygous groups and by its retrospective nature. In addition, the study population was a typical sample
8of a clinical trial for MCI who are in general healthier than the general population due to strict
9inclusion/exclusion criteria. The study was composed mainly of non-Hispanic Caucasians which also
10limits the generalizability of our findings. Despite these limitations, our findings are in agreement with
11results reported recently by Han *et al.* with the use of rivastigmine [16]. In their case-only study, they
12found that the BChE-K allele was a significant predictor of poor rivastigmine response in Korean AD
13dementia patients [16]. Hence, poor response associated with the BChE-K variant might be seen with all
14medications from the AChEI class and not just with donepezil as we found here. Yet, other case-only
15pharmacogenetic studies failed to detect an interaction between BChE K-genotype and AChEI effect on
16cognitive function response in late onset AD (LOAD) [11, 15]. It is important to point out that both the
17sample size and the duration of these LOAD studies were much smaller than the cohort we analyzed here
18[Table 2], which might explain their negative findings. However, the largest of the three did see an effect
19of genotype on response to therapy but the authors could not determine whether the treatment was
20deleterious in BChE-K demented subjects since they did not have a placebo group [16]. In their study,
21they reported a lower response rate among APOE4 carriers treated with rivastigmine, with no difference
22among APOE4 non-carriers [16].

23 In conclusion, our data indicate that rs1803274 is a pharmacogenetic marker of donepezil
24response in MCI. BChE genotyping of locus rs180327 is valuable in detecting individuals who are likely
25to demonstrate a faster cognitive decline in MCI if treated with donepezil, and principally in those
26carrying the APOE4 allele. Thus, this opens up the discussion of the off-label use of AChEIs in MCI and

1 offers the prospect of rationalized pharmacogenetic approaches for personalized-medicine in the treatment
2 of individuals with MCI when there is no alternative to AChEIs. We conclude that BChE-K genotype
3 should be routinely tested and that donepezil should not be prescribed off-label to BChE K-variant
4 carriers, especially in APOE4 carriers, as it leads to disease exacerbation and faster cognitive decline in
5 MCI.

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11 **Author Contributions**

12 Conception and design of the study: S.S, X.L, K.D.T, J.I.R, R.A.R, P.S.A, L.G.A. Acquisition and
13 analysis of data: S.S, L.C, X.L, K.T, J.R, R.A.R, P.S.A, L.G.A. Drafting the manuscript or figures: S.S,
14 L.C, X.L, K.T, J.I.R and L.G.A.

15 **Potential Conflicts of Interest**

16 Nothing to report.

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1Figure legends

2**Figure 1.** Effect of BChE-K genotype on cognitive decline in control (**A and C**) vs. donepezil treated (**B**
3**and D**) individuals at the end of the 36-months trial. Box plot of the mean changes of MMSE (**A and B**)
4and CDR-SB (**C and D**) by genotype and by treatment groups. A significant association is found between
5BChE K-genotype and response to donepezil (**B and D**).

6

7**Figure 2.** Mean changes in MMSE (**A and B**) and CDR-SB (**C and D**) scores over time by BChE-K
8genotype and by treatment groups. BChE polymorphisms rs1803274 define sub-populations with
9different response to donepezil therapy in MCI subjects. The long-term treatment effects for both MMSE
10and CDR-SB changes are significantly different among BChE genotype groups (**B and D**). No significant
11interaction between BChE genotype and cognitive decline in treatment controls (**A and C**).

12

13**Figure 3.** Mean changes in MMSE (**A and B**) and CDR-SB (**C and D**) scores over time by BChE-K
14genotype and by treatment groups in APOE4 carriers. The long-term effect of donepezil for both MMSE
15and CDR-SB changes are significantly different among BChE genotype at loci rs1803274 in APOE4
16carriers (**B and D**). No significant interaction between BChE genotype and cognitive decline in untreated
17APOE4 carrier subjects (**A and C**).

18

Table 1. Patients' demographic information at baseline

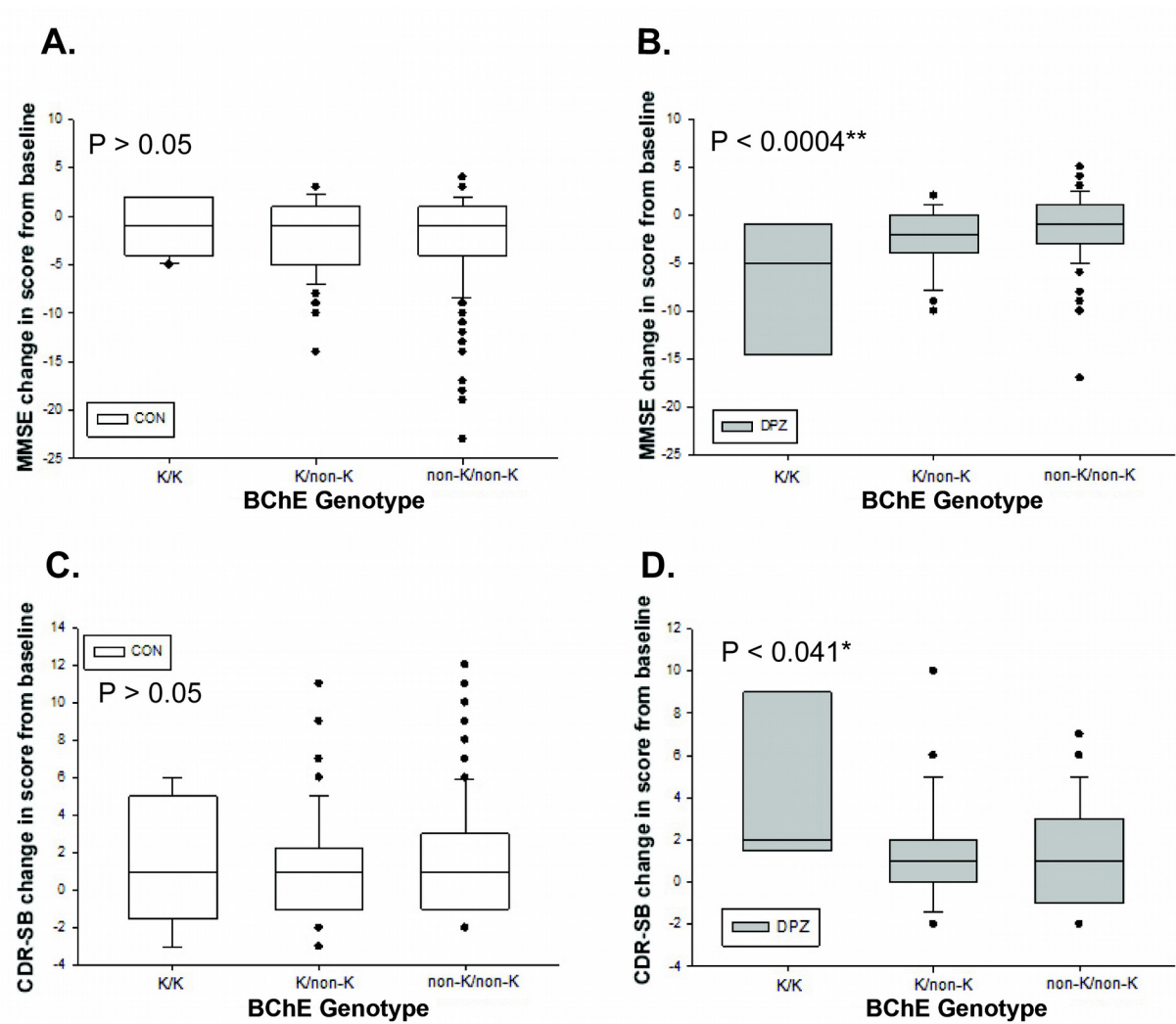
	Donepezil treated	Donepezil naive	P
N (%)	193 (33.6%)	381 (66.4%)	
Age (SD)	73.19 (6.80)	72.77 (8.26)	0.74
Male (%)	105 (54.4%)	204 (53.5%)	0.86
APOE4 (%)	87 (45.1%)	183 (48.0%)	0.53
MMSE (SD)	27.3 (1.8)	27.3 (1.8)	
CDR-SB (SD)	1.8 (0.8)	1.8 (0.7)	

Table 2. BChE Pharmacogenetic studies of AChEI response in Late Onset Alzheimer's Disease

Study	Ethnicity	AChEI	Treatment duration	Placebo control	Outcome variables	N per BChE genotype			P value
						K0	K1	K2	
Scacchi et al. 2008 [11]	Caucasian	Donepezil	15-months	No	MMSE	64	32	4	>0.05
		Rivastigmine			MMSE	41	28	0	> 0.05
Han et al. [16]	Asian	Rivastigmine alone or with memantine	16 weeks	No	MMSE ADAS-cog	111 111	35 35		>0.05 <0.001**
Blesa et al. [15]	Caucasian	Donepezil Rivastigmine	2 years	No	MMSE MMSE	43 33	19 19		>0.05 >0.05

*K genotype: K0 denotes the absence of the K allele, K1 is for the heterozygous carriers and K2 for the homozygous carriers.

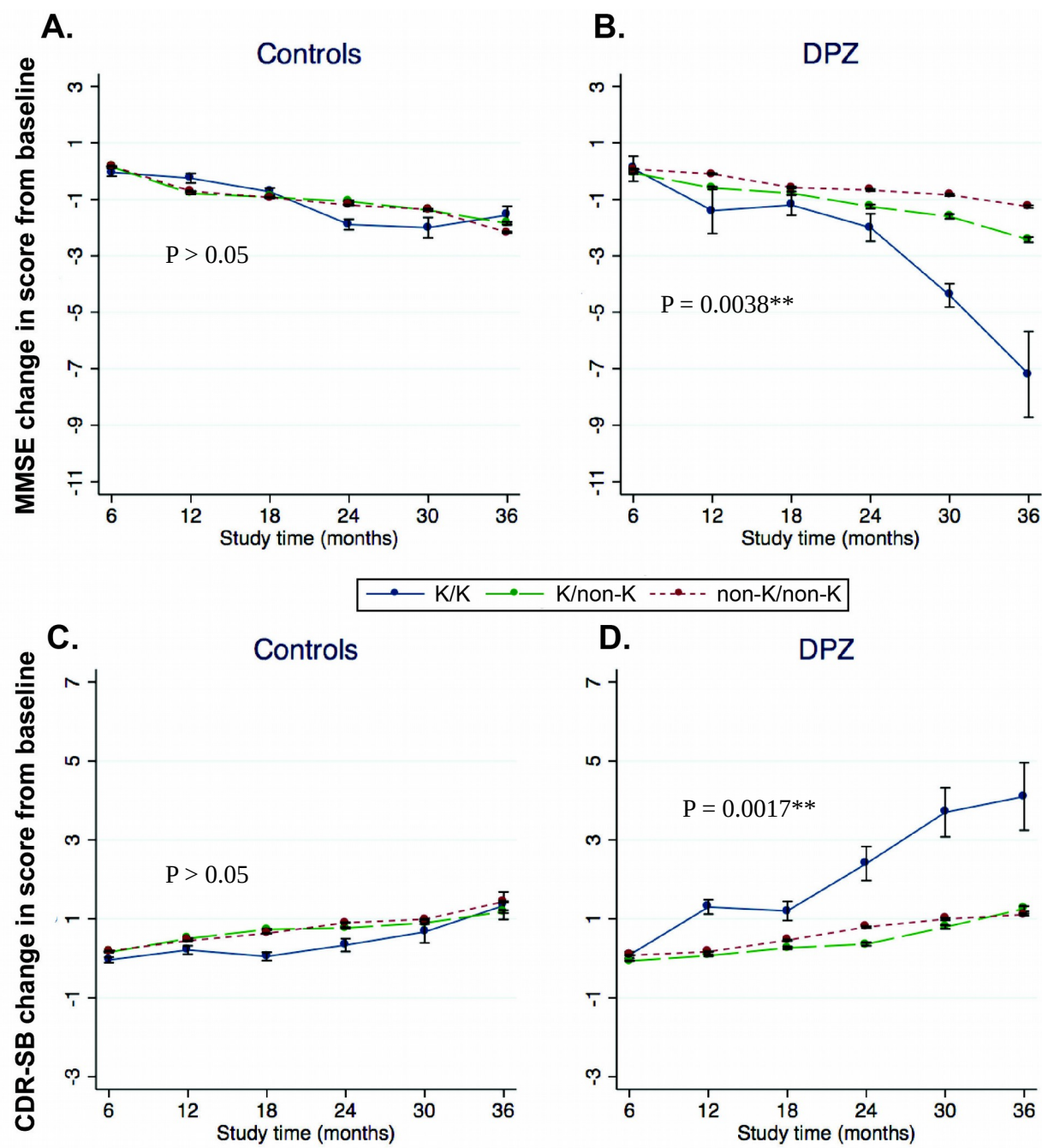
1Figure 1



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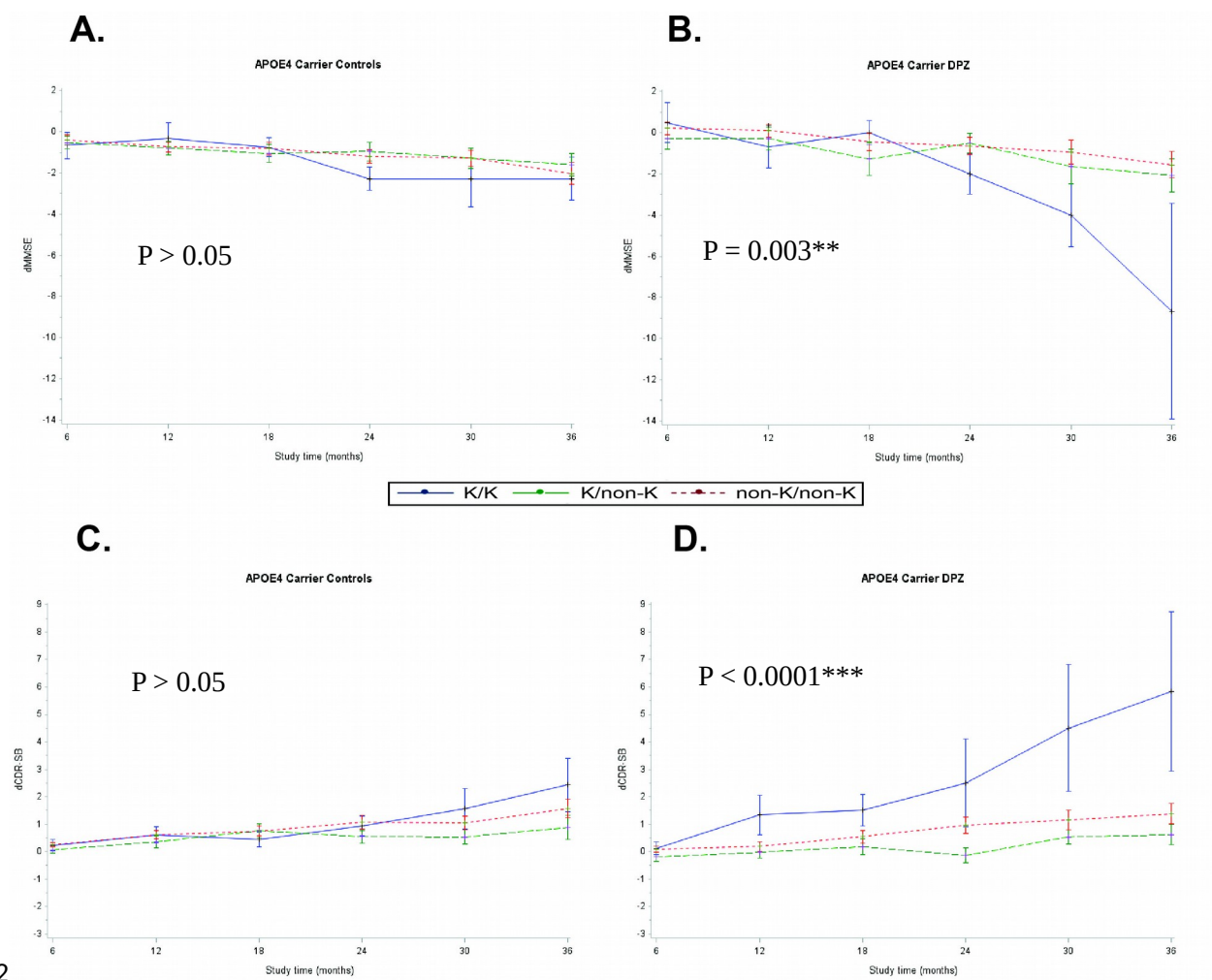
1Figure 2.



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1Figure 3.



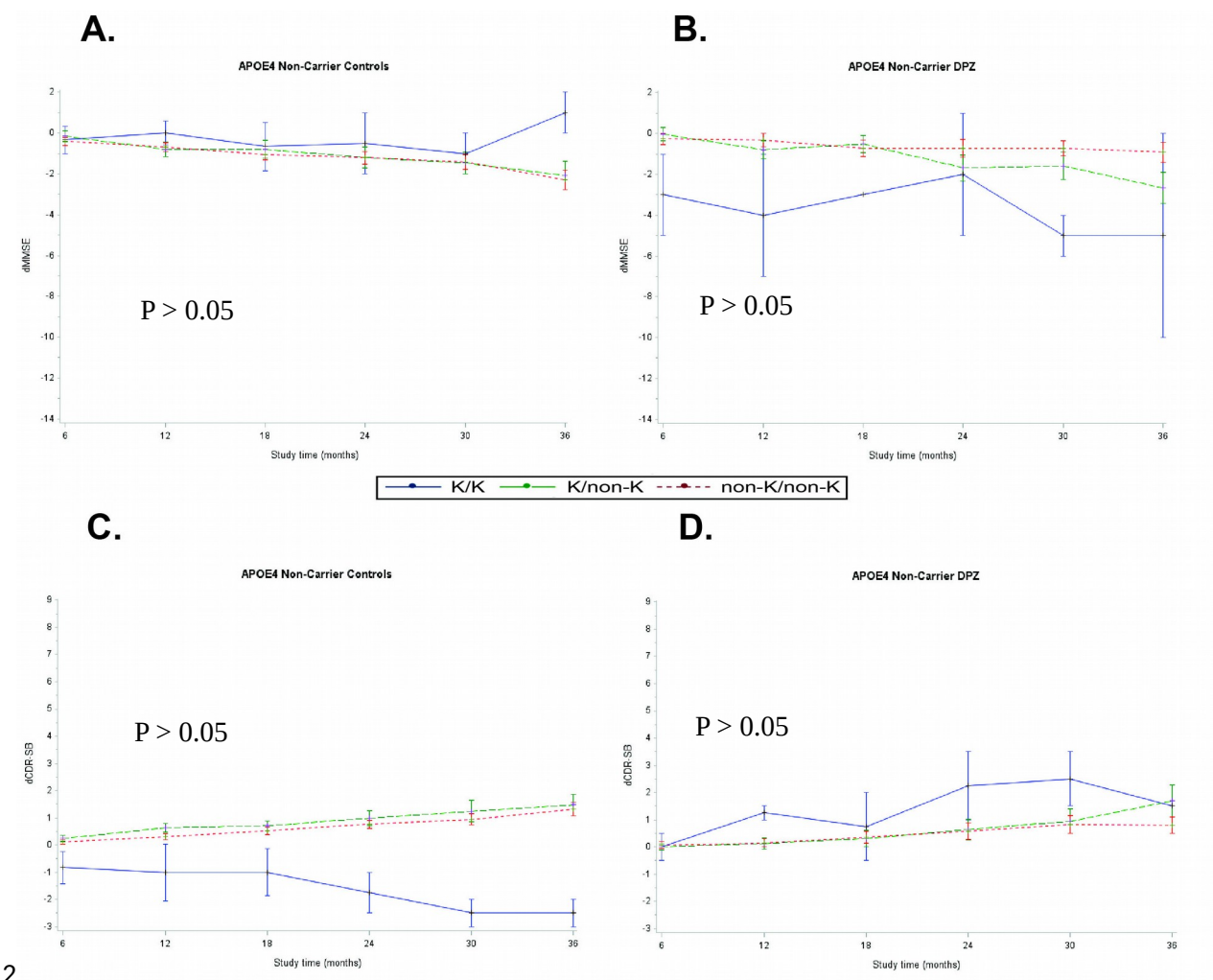
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1Supplementary figure legend

2 **Figure S1.** Mean changes in MMSE (**A and B**) and CDR-SB (**C and D**) scores over time by BChE-K
3genotype and by treatment groups in APOE4 non-carriers. In APOE4 non-carriers, there was no
4significance difference in MMSE and CDR-SB changes between donepezil treated subjects and controls
5among BChE genotypes at loci rs1803274.

1Supplementary Figure S1.



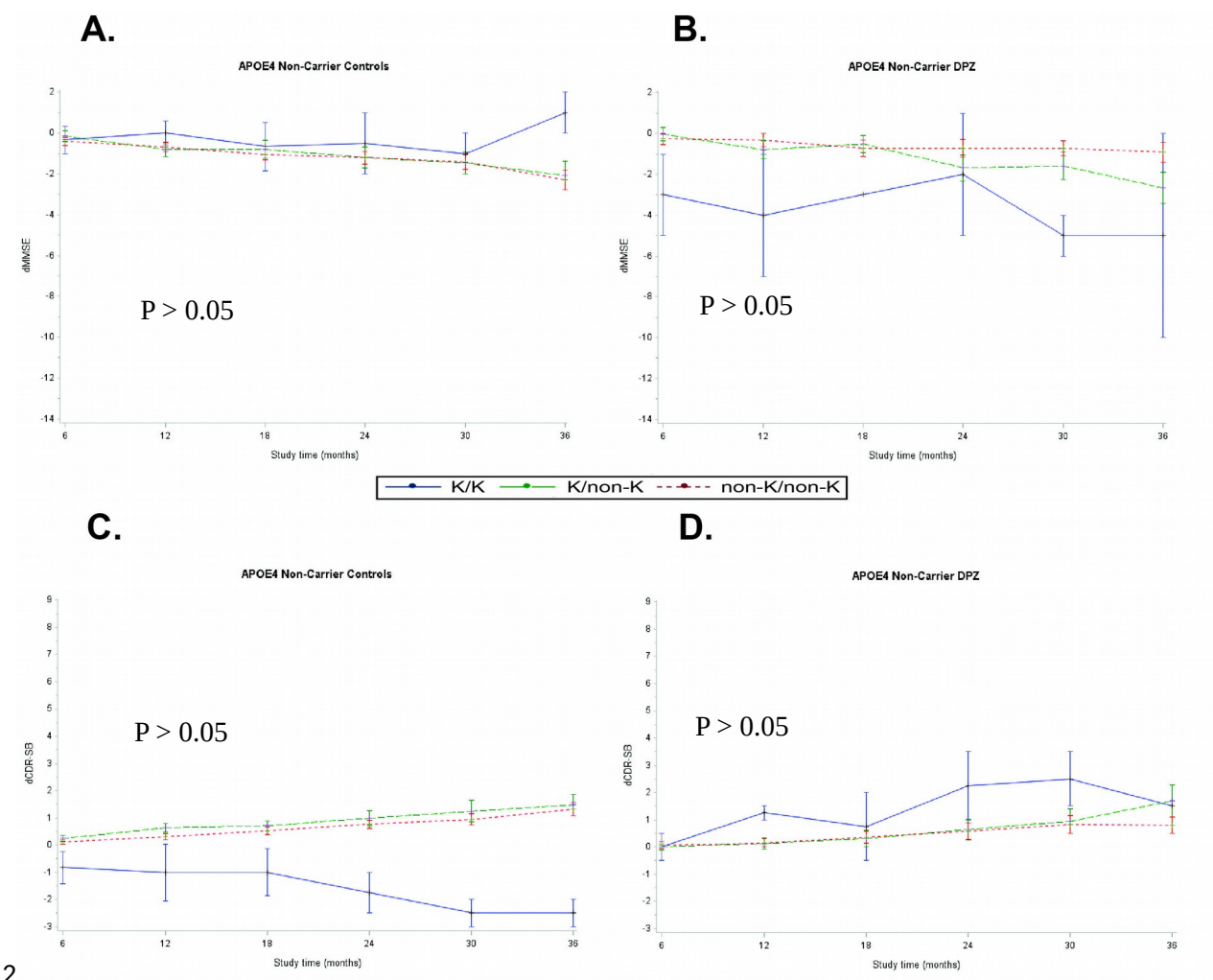
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