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Analysis of association between Alzheimer disease and the K variant of butyrylcholinesterase (BCHE-K)

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Abstract

Butyrylcholinesterase (BCHE) is an enzyme expressed in most human tissues. Recently, an increased odds of carrying the K variant of BCHE (BCHE-K) was reported among Alzheimer disease (AD) cases as compared with controls. We tested our data set of 245 sporadic AD cases and 241 controls for an association between BCHE-K, APOE4, and AD using logistic regression and chi-square analyses. The sib transmission disequilibrium test (S-TDT) was also used to test for differences in BCHE-K allele frequencies between 163 discordant sib-pairs selected from multiplex AD families. No statistically significant differences were noted between BCHE-K case and control allele frequencies even after stratifying by APOE4 status. S-TDT analysis between the BCHE-K variant and AD was also not significant ($P = 0.52$). We conclude that BCHE-K is not a major genetic risk factor for AD in our study population.

Keywords: Butyrylcholinesterase gene; Apolipoprotein E; Alzheimer disease; Transmission disequilibrium test; Association; Linkage disequilibrium

Alzheimer disease (AD), the leading cause of dementia in Americans aged 65 and older, is a progressive brain disorder, which occurs gradually and results in memory loss, unusual behavior, personality changes, and an irreversible decline in thinking abilities [11]. To date, four genes which account for approximately half of the genetic risk of AD [5] have been identified: amyloid precursor protein (APP) [6]; presenilin 1 (PS1) [18]; presenilin 2 (PS2) [9,13]; and apolipoprotein E (APOE) [17]. Mutations in APP, PS1, and PS2 cause the early-onset Mendelian form of AD while APOE is considered a susceptibility gene, where the APOE4 allele increases risk of AD in a dose-dependent fashion [3]. Although a number of additional genes have been examined as possible AD risk factors, findings have been inconsistent, leaving the remaining genetic effect in AD unexplained.

Two recent case-control studies [8,15] reported an increased odds of late-onset AD cases carrying the K variant of the butyrylcholinesterase gene (BCHE-K) when compared to control subjects. The association was strongest (OR = 15.00, OR = 14.40, respectively) among subjects who were carriers of the APOE4 allele. Another study [4] identified a statistically significant association (OR = 1.89) between BCHE-K and AD among subjects who did not carry the APOE4 allele. Additional studies [2,7,14,20] failed to confirm a positive association between BCHE-K and AD.

The purpose of the present study was to determine if an association between BCHE-K and AD exists in our data set using two different analysis methods: the traditional unmatched case-control study design and a newly developed family-based association method, the sib transmission disequilibrium test (S-TDT) [21].

The study population for the case-control analyses consisted of 245 AD cases with no reported family history of AD or dementia and 241 unrelated controls. Cases were

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Table 1
BCHE-K allele frequencies among sporadic cases and controls[a]

APOE4 status	Age category	Cases (%)		Controls (%)		*P*-value*
All subjects	All ages	18.8	(92/490)	23.0	(111/482)	0.10
	<65	18.4	(28/152)	28.1	(27/96)	0.07
	65–75	17.2	(43/250)	21.7	(62/286)	0.19
	>75	23.9	(21/88)	22.0	(22/100)	0.76
APOE4 (+)	All ages	18.0	(59/328)	25.4	(32/126)	0.08
	<65	20.2	(19/94)	29.4	(10/34)	0.27
	65–75	15.8	(29/184)	24.4	(19/78)	0.10
	>75	22.0	(11/50)	21.4	(3/14)	0.96
APOE4 (−)	All ages	20.4	(33/162)	22.2	(79/356)	0.64
	<65	15.5	(9/58)	27.4	(17/62)	0.11
	65–75	21.2	(14/66)	20.7	(43/208)	0.93
	>75	26.3	(10/38)	22.1	(19/86)	0.61

[a] Numbers in parentheses = number of BCHE-K alleles/total number of alleles; number of alleles is twice the number of subjects. **P*-value for χ^2 test of difference in BCHE-K allele frequency between cases and controls.

ascertained through the Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) at Duke University and diagnosed with probable or possible AD using the NINCDS-ADRDA clinical diagnostic criteria [10]. The controls exhibited no signs of dementia and were spouses of clinically ascertained AD or dementia patients. For cases, the mean age of onset was 67.9 ± 8.3 years (range 40–85) and the mean age of examination was 72.7 ± 8.0 (range 42–93). The mean age of exam for controls was 69.7 ± 7.3 years (range 37–86). Fifty-eight percent of cases and 52% of controls were female. All cases and controls were Caucasian. The data set for the S-TDT contained 163 sibships consisting of one unaffected and one to two affected siblings from previously described independent, multiplex, Caucasian late-onset (>60) AD families [1,12]. S-TDT cases were diagnosed as above [10].

Subjects' DNA was obtained by standard methods [12]. Appropriate informed consent was obtained from all participants or their legal representatives. All subjects' DNA was genotyped for BCHE-K and APOE. BCHE-K genotyping was performed as previously described [19], with the following modifications: the digested DNA was separated by electrophoresis on an 8% acrylamide gel, and the gels were stained with SYBR Gold (Molecular Probes) and viewed on a fluoroimager (Molecular Dynamics). APOE genotyping was performed as previously reported [17]. Subjects with one or two BCHE-K alleles were considered BCHE-K carriers (BCHE-K(+)), while subjects with no BCHE-K alleles were considered non-carriers (BCHE-K(−)). Similarly, subjects with at least one APOE4 allele were classified as APOE4(+); subjects without an APOE4 allele were classified as APOE4(−). All data were stored in the PEDIGENE® database system.

BCHE-K allele and carrier frequencies were calculated for cases and controls and the genotype frequencies tested for Hardy–Weinberg equilibrium. We used the chi-square test of association to test for allele and carrier frequency differences between cases and controls, for all ages

Table 2
BCHE-K carrier frequencies among sporadic cases and controls and odds ratios for the association between BCHE-K and AD[a]

APOE4 status	Age category	Cases (%)		Controls (%)		*P*-value*	Odds ratio	95% CI**
All subjects	All ages	34.7	(85/245)	41.1	(99/241)	0.14	0.75***	(0.50, 1.13)
	<65	35.5	(27/76)	45.5	(22/48)	0.25		
	65–75	32.0	(40/125)	39.2	(56/143)	0.22		
	>75	40.9	(18/44)	42.0	(21/50)	0.91		
APOE4 (+)	All ages	32.9	(54/164)	46.0	(29/63)	0.07	0.58****	(0.32, 1.04)
	<65	38.3	(18/47)	52.9	(9/17)	0.30		
	65–75	28.3	(26/92)	43.6	(17/39)	0.09		
	>75	40.0	(10/25)	42.9	(3/7)	0.89		
APOE4 (−)	All ages	38.3	(31/81)	39.3	(70/178)	0.87	0.96****	(0.55, 1.66)
	<65	31.0	(9/29)	41.9	(13/31)	0.38		
	65–75	42.4	(14/33)	37.5	(39/104)	0.61		
	>75	42.1	(8/19)	41.9	(18/43)	0.99		

[a] Numbers in parentheses = number of BCHE-K carriers /total number. **P*-value for χ^2 test of difference in carrier frequency between cases and controls. **CI = confidence interval; ***adjusted for age (continuous), sex, and APOE4; ****adjusted for age (continuous) and sex.

combined, and by age (<65, 65–75, >75). These analyses were repeated with stratification for APOE4(+)/(−) status. Logistic regression [16] was used to model the association between AD and BCHE-K. The dependent variable in the model was AD; the major independent variable of interest was BCHE-K carrier status (BCHE-K(+), BCHE-K(−) (reference)). Additional independent variables included in the model were the continuous variable age (onset for cases, exam for controls); sex (female, male (reference)); and APOE4 carrier status (APOE4(+), APOE4(−) (reference)). This model was also analyzed stratified by APOE4 status. To obtain an overall significance level of $\alpha = 0.05$, we corrected for 24 chi-square comparisons using the Bonferroni method, declaring individual tests significant at the level of $\alpha = 0.002$.

The S-TDT was performed on 163 discordant (affected/unaffected) sibling pairs. Only one pair per family was included to ensure that the S-TDT be a valid test of association. Unaffecteds younger than 60 years of age were excluded to reduce potential misclassification of asymptomatic AD carriers. The S-TDT was also performed for the subset of discordant sib pairs ($n = 81$ pairs) in which both members of the pair had at least one APOE4 allele. Reported P-values were calculated using the Z score method [21].

There was no evidence that the BCHE-K genotypes were in Hardy–Weinberg disequilibrium for cases ($P = 0.25$) or controls ($P = 0.39$). Results from the case-control analyses are presented in Tables 1 and 2. Table 1 lists the frequency of the BCHE-K allele among cases and controls and gives the chi-square p-value for BCHE-K allele frequency differences between these two groups. These results are reported for the overall data set and the APOE carrier and non-carrier subsets, for all ages combined and by age category. The BCHE-K allele frequency was 19, 18 and 20% among all cases, APOE4(+) cases, and APOE4(−) cases respectively; and 23, 25 and 22% among all controls, APOE4(+) controls, and APOE4(−) controls, respectively. There were no statistically significant differences in BCHE-K allele frequencies between cases and controls in the overall data set or in either of the subsets. However, for all subjects and for APOE4(+) subjects, the BCHE-K allele frequency was lower among cases than controls for all age categories except age >75. With Bonferroni correction for multiple comparisons, the observed P-values were much greater than $P = 0.002$, the P-value below which it would be appropriate to declare statistical significance at the overall level of $\alpha = 0.05$. No trend of BCHE-K allele frequency being lower among cases than controls was noted among APOE4(−) subjects.

Table 2 lists BCHE-K carrier frequencies among cases and controls. The carrier results were similar to the allele frequency results. There were no statistically significant differences in BCHE-K carrier frequencies between cases and controls. However, the point estimate for the BCHE-K carrier frequency was lower among cases than controls for all subjects in every age category and for APOE4(+) subjects in every age category in every age group; but not for APOE4(−) subjects. Again, with Bonferroni correction this difference was far from the P-value of 0.002 required to achieve an $\alpha = 0.05$ level of statistical significance. Odds ratio estimates obtained from logistic regression for the association between AD and being a BCHE-K carrier are also presented in Table 2. These odds ratios were adjusted for age, sex and APOE4 carrier status. Adjustment for APOE4 carrier status was accomplished by inclusion of the variable in the overall model, or by stratification on +/− status. None of the logistic regression results were statistically significant.

The S-TDT did not detect a statistically significant difference ($\chi^2 = 0.41$, $P = 0.52$) in the BCHE-K allele frequency between affected and unaffected siblings ($n = 163$ pairs). Subsetting the S-TDT data set to discordant sib pairs where each sib carried at least one APOE4 allele ($n = 81$ pairs) also resulted in no statistically significant difference in the BCHE-K allele frequencies ($\chi^2 = 0.68$, $P = 0.41$). In contrast, the S-TDT analysis of APOE in this same data set was highly significant ($\chi^2 = 30.81$, $P < 0.0001$).

There were no statistically significant differences in BCHE-K allele frequencies between cases and controls in our data set; nor were there any statistically significant differences in the percent of cases and controls that were BCHE-K carriers, even after stratifying by APOE4 carrier status. Although not statistically significant, BCHE-K allele frequencies were consistently lower among cases than controls for all subjects and APOE4(+) subjects younger than 75 (Table 1); similarly, the BCHE-K carrier frequency was consistently lower in cases than in controls among all subjects and APOE4(+) subjects for all age categories (Table 2). Similar trends have been previously reported by other investigators ([4] (community sample), [7,20]).

The lack of corroboration between the above studies and other BCHE-K and AD case-control studies [2,8,14,15] could reflect true genetic differences, as the study populations were drawn from several different continents and at least four different countries (Finland, Toronto, UK, USA). However, dissimilarities in study results could also be due to differences in sampling strategies. Age differences between the populations studied probably did not play much of a role in study result differences because most studies accounted for age differences by stratification or through inclusion of age in a logistic regression model. It is difficult to assess how racial differences affected study results as many studies did not state their subjects' race. Case and control ascertainment methods may have played a role in study differences. Cases were ascertained from both clinics and communities, with some clinic cases sampled as part of a clinical trial and some not. Similarly, controls were recruited from both community and clinic-associated (e.g. spouses of patients) groups. The way in which different ascertainment methods could influence results is clearly demonstrated by one study [4] which used the same community control group with two

separate clinic and community case populations. For APOE4(−) subjects, this study reported a statistically significant increased odds (OR = 1.89) of AD clinic cases being BCHE-K carriers when compared with community controls; in contrast, a decreased, though not statistically significant, odds (OR = 0.42) of carrying a BCHE-K allele was reported when comparing community-ascertained cases with the identical control group.

Our study adds to the other BCHE-K and AD association studies [2,4,7,8,14,15,20] in that we used the recently developed S-TDT as an additional test of association. A common problem in the case-control study design is the detection of spurious associations resulting from population stratification. Using the S-TDT is advantageous in that it overcomes the problem of poorly ethnically matched cases and controls by using the unaffected sib as a control for the affected sib of a pair.

Using the S-TDT, we did not find evidence of an association between BCHE-K and AD. However, applying the S-TDT to the APOE data in the same study population resulted, as expected, in a highly significant P-value ($P < 0.0001$). We therefore conclude that if there was association between BCHE-K and AD that was at least as strong as or stronger than the association between APOE and AD, we should have had sufficient power to detect it using the S-TDT.

Sample size may have played a role in the failure of some studies to find statistically significant results. Our overall case-control sample size ($n = 486$) was larger than three [7,8,15] of the four studies [4,7,8,15] that reported statistically significant results and all of the studies [2,14,20] that did not report statistically significant results. Power studies indicate that our overall data set should have had at least 80% power to detect a positive OR = 1.75 or greater, or a protective OR = 0.57 or smaller, assuming an $\alpha = 0.05$ level of significance and carrier frequency among all controls of 0.41 (Table 2). Therefore, we have high power to detect an association between AD and carrying a BCHE-K allele as strong as that reported by Lehmann et al. [8] (OR = 2.30) in our data set. Similarly for only APOE4(+) subjects, our sample size ($n = 164$ cases, 63 controls) should have had sufficient power to detect a positive OR = 2.50 or greater, or a protective OR = 0.40 or smaller, applying the same assumptions as above, but with a carrier frequency among APOE4(+) controls of 0.46 (Table 2). Therefore, our study should have had high power to detect associations as strong as those reported by Lehmann et al. [8] and Sandbrink et al. [15] (OR = 15.00, OR = 14.40, respectively) among their later onset APOE4(+) subjects.

In conclusion, we find no significant evidence for an association between BCHE-K and AD using either the traditional case-control methodology or the family-based association method, the S-TDT. We therefore conclude that BCHE-K is not a major genetic risk factor for AD in our study population.

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