Chemico-Biological Interactions 175 (2008) 115–118

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Association of variants of the −116 site of the butyrylcholinesterase *BCHE* gene to enzyme activity and body mass index

Lupe Furtado-Alle, Fabiana A. Andrade, Kelly Nunes, Liya R. Mikami, Ricardo L.R. Souza, Eleidi A. Chautard-Freire-Maia*

Department of Genetics, Federal University of Paraná, Brazil

ARTICLE INFO

Article history:
Available online 29 April 2008

Keywords:
BCHE gene variants
Butyrylcholinesterase activity
Body mass index
−116A mutation
K mutation

ABSTRACT

Butyrylcholinesterase (BChE) is coded by the *BCHE* gene that presents four exons. The non-codifying exon 1 presents two variants *−116G* and *−116A*, being *−116A* preferentially in *cis* conformation with the *539T* variant (*K*) of exon 4 which was associated with lower BChE activity and lower body mass index (BMI) variance. This study analyzed the frequency of −116 variants and the relation of genotypes *−116GG;539AA*, *−116GG;539AT* and *−116GA;539AT* with BChE activity and with BMI in Euro-Brazilian blood donors. The frequency of *−116A* was significantly higher (18.9%) in the low BChE activity group when compared to obese (8.6%) and normal BMI (9.3%) groups. In obese and non-obese groups, the *−116GA;539AT* genotype showed significantly lower mean BChE activity when compared to the *−116GG;539AA* genotype and in obese individuals the *−116GA;539AT* genotype also showed lower BChE activity than the *−116GG;539AT* genotype. In a sample selected independently of BMI, the *−116GA;539AT* genotype showed significantly higher BMI variance (21.75) when compared to *−116GG;539AA* (12.14) and to *−116GG;539AT* (13.43) genotypes, indicating that the association with higher BMI variance only occurs in the presence of the *−116A* variant. In the obese sample, the *−116GG;539AT* genotype presented mean (32.1 ± 0.3) and variance (2.3) of BMI significantly lower than those found in the *−116GG;539AA* (33.0 ± 0.3 and 9.9, respectively) and *−116GA;539AT* (33.7 ± 0.7 and 12.2, respectively) genotypes. These data show that: (1) the *K* (*539T*) variant alone is not associated with decreased BChE activity, being the 5′ UTR *−116A* variant necessary for this decrease, probably by affecting transcription and/or translation of the *BCHE* gene; (2) samples with different BMI distributions present different relationships between *BCHE* genotypes and BMI, reinforcing the hypothesis of a role for the *BCHE* gene in BMI determination.

1. Introduction

Butyrylcholinesterase (BChE) is coded by the *BCHE* gene that presents four exons. The most frequent non-usual variant in the coding region is the *K* (*539T*) variant located in exon 4 (SNP: G/A; rs1803274; *p.A539T*; *1615* nt) which was associated with lower BChE activity [1]. The non-codifying exon 1 presents two variants at −116 nt (SNP: G/A; rs1126680), being *−116A* preferentially found in *cis* with the *K* variant [2]. The *−116A* variant was first identified with a frequency of 8.0% [2] and a frequency of 18.4% was reported for the *539T* variant in Euro-Brazilians [3]. The *K* variant was associated with late onset and a slowing of the progression of Alzheimer's disease [4–6] and with higher body mass index (BMI) variance [7].

In the present report the genotype nomenclature considered that Glu 1 is the N-terminal aminoacid of the mature BChE protein and nucleotide 1 corresponds to the first nucleotide in the codon for Glu 1 [8]. The base code

* Corresponding author at: P.O. Box 19071, 81531-990; Curitiba, PR, Brazil. Tel.: +55 41 33611553; fax: +55 41 32446344.
E-mail address: eleidi@ufpr.br (E.A. Chautard-Freire-Maia).

0009-2797/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2008.04.019

Table 1
Butyrylcholinesterase activity means ± S.E. in obese and control groups, showing comparisons between genotypes in each group

Exons 1 and 4			
Obese ($N = 140$)	N	Genotype frequency, (%) ± S.E.	BChE activity (KU/L), means ± S.E.
−116GG;539AA	92	65.72 ± 4.01	6.60 ± 2.69
−116GG;539AT	21	15.00 ± 3.02	7.29 ± 3.94
−116GA;539AT	21	15.00 ± 3.02	5.10 ± 2.14
−116GG;539TT	03	2.14 ± 1.22	6.59 ± 3.53
−116GA;539TT	03	2.14 ± 1.22	4.97 ± 0.38
Comparisons of mean BChE activity			t-test (p)
−116GG;539AA × *−116GG;539AT*			0.76 (0.45)
−116GG;539AA × *−116GA;539AT*			2.39 (0.02)
−116GG;539AT × *−116GA;539AT*			2.24 (0.03)
Control ($N = 140$)	N	Genotype frequency, (%) ± S.E.	BChE activity (KU/L), means ± S.E.
−116GG;539AA	86	61.43 ± 4.11	5.03 ± 1.58
−116GG;539AT	26	18.57 ± 3.29	4.49 ± 1.26
−116GA;539AT	18	12.86 ± 2.83	3.89 ± 0.93
−116GG;539TT	03	2.14 ± 1.22	3.93 ± 0.51
−116GA;539AA	03	2.14 ± 1.22	4.20 ± 0.62
−116GA;539TT	03	2.14 ± 1.22	4.03 ± 0.61
−116AA;539TT	01	0.71 ± 0.71	2.90
Comparisons of mean BChE activity			t-test (p)
−116GG;539AA × *−116GG;539AT*			1.62 (0.11)
−116GG;539AA × *−116GA;539AT*			4.11 (0.0002)
−116GG;539AT × *−116GA;539AT*			1.71 (0.09)

was used for genotypes of the −116 site whereas the aminoacid code was used for variants of exon 4 of the *BCHE* gene.

Considering the linkage disequilibrium between variants of exons 1 and 4 of the *BCHE* gene, this study compared genotypes of these two exons in relation to BChE activity and BMI and estimated the frequency of −116 variants in Euro-Brazilians. As obese population samples usually show higher mean BChE activity when compared to samples with normal BMI, the study of these two kinds of samples was considered of importance and it was shown that the *−116A* variant affects BChE activity in both samples.

2. Material and methods

2.1. Population samples

Analyses concerning mean BChE activity comparisons and *−116A* frequency calculation used three sub-samples of Euro-Brazilian male blood donors: 140 obese individuals (BMI ≥ 30); 140 control individuals (20 ≤ BMI < 25) paired by age to the obese blood donors; and 106 individuals selected only by low BChE activity (≤4.0 KU/L).

Analyses concerning BMI were preformed in two blood donors sub-samples: (1) the same sample which showed association between the *K* variant and higher BMI variance [7], selected on the basis of exon 4 genotypes: *539AT* ($N = 220$) and *539AA* ($N = 220$), paired by sex, age and ethnic group; (2) the same sub-sample of obese blood donors used for BChE activity analyses in which another four obese individuals were included. In those selected by exon 4 genotype, two individuals with the *−116GA;539AA* genotype were excluded from the analysis and the three considered genotypes (*116GG;539AA*, *−116GG;539AT*, *−116GG;539TT*) do not differ in the respective mean ages (31.4 ± 0.7S.E.; 31.7 ± 0.9S.E.; 31.3 ± 1.2S.E.), frequencies of males (83.9 ± 2.5%; 86.0 ± 3.0%; 80.2 ± 4.2%) and frequencies of Euro-Brazilians (95.9 ± 1.3%; 93.7 ± 2.2%; 97.8 ± 1.5%). Obese individuals of these three genotypes do not differ in mean age: 35.9 ± 1.0S.E.; 35.7 ± 1.8S.E.; 39.2 ± 2.4S.E., respectively.

This research was approved by the ethical committee.

2.2. DNA and plasma analysis

DNA was extracted by salting out [9] and PCR for exon 1 of the *BCHE* gene used primers designed for the present study: E1F (5′-CTG CTG CCA ACT CTC GCG AG-3′) and E1R (3′-CGA AGG TGT AAA TTC AGA GC-5′) from −268 to 27 nt of the 5′ end of intron 1. Primers P43 and P45 [10] were used for exon 4 amplification. Amplification cycles were: (1) 80 °C/min.; (2) 94 °C/min.; (3) 48 °C/min.; (4) 72 °C/min.; (5) repeat 35 times from steps 2 to 4; (6) 72 °C/10 min.

Single strand conformation analysis was used for variant detection. Polyacrylamide gel (170 mm × 160 mm × 0.8 mm; TBE 1X; 10% and 8% polyacrylamide concentration for exons 1 and 4, respectively and 29:1 stock solution) electrophoresis at 4 °C (35 mA and 5 h for exon 1; 15 mA and 22 h for exon 4) was followed by silver staining of the gel [11].

Sequencing of exon 1 (primers E1R and E1F) was done for 20 samples with the Big Dye Terminator kit (Applied Biosystems) in an ABI 377 sequencer.

Plasma BChE activity was determined with the use of propionylthiocholine as substrate at 25 °C [12].