Genetic Variants of Serum Butyrylcholinesterase in Chilean Mapuche Indians

M. Acuña,[1]* L. Eaton,[2] N.R. Ramírez,[1] L. Cifuentes,[1] and E. Llop[1]

[1]*Genetic Program ICBM, Faculty of Medicine, University of Chile, Santiago 1, Chile*
[2]*Science Faculty Adolfo Ibáñez University, Santiago, Chile*

KEY WORDS plasma butyrylcholinesterase; *BCHE* locus; genetic variants; Mapuche Indians

ABSTRACT We estimated the frequencies of serum butyrylcholinesterase (BChE) alleles in three tribes of Mapuche Indians from southern Chile, using enzymatic methods, and we estimated the frequency of allele *BCHE*K* in one tribe using primer reduced restriction analysis (PCR-PIRA). The three tribes have different degrees of European admixture, which is reflected in the observed frequencies of the atypical allele *BCHE*A*: 1.11% in Huilliches, 0.89% in Cuncos, and 0% in Pehuenches. This result is evidence in favor of the hypothesis that *BCHE*A* is absent in native Amerindians. The frequencies of *BCHE*F* were higher than in most reported studies (3.89%, 5.78%, and 4.41%, respectively). These results are probably due to an overestimation of the frequency of allele *BCHE*F*, since none of the 20 BCHE UF individuals (by the enzymatic test) individuals analyzed showed either of the two DNA base substitutions associated with this allele. Although enzymatic methods rarely detect the presence of allele *BCHE*K*, PCR-PIRA found the allele in an appreciable frequency (5.76%), although lower than that found in other ethnic groups. Since observed frequencies of unusual alleles correspond to estimated percentages of European admixture, it is likely that none of these unusual alleles were present in Mapuche Indians before the arrival of Europeans. Am J Phys Anthropol 121: 81–85, 2003.

Previous papers in this series described in some detail the population structure and a variety of genetic attributes of Chilean Indians (Goedde et al., 1984; Acuña et al., 1994; Llop et al., 1993; Llop, 1996; Harb et al., 1998). Today, surviving Mapuches, comprising several more or less independent tribes (Picunches, Chiquillanes, Huilliches, Puelches, Cuncos, and Pehuenches), are no longer full-blooded aborigines. The estimated introduction of European genes (principally Spanish) is reported to be as high as 25–30% in some areas (Matson et al., 1967). We present here the results of examining three of the Mapuche groups with respect to their genetic response to the drug suxamethonium.

Suxamethonium is a drug commonly used in anesthetics as a short-acting muscle relaxant during surgery. Suxamethonium sensitivity is occasionally manifested as prolonged apnea. Genetic variants of normal serum butyrylcholinesterase (acylcholine acylhydrolase, EC 3.1.1.8) which fail to hydrolyze this drug have occasionally been detected. The first genetic variant discovered was called "atypical" (*BCHE*A*), whereas the normal allele was termed "usual" (*BCHE*U*). Since 1961, a number of further variant alleles have been identified, such as *BCHE*F*, *BCHE*K*, *BCHE*S*, and *BCHE*J* (Kalow, 1992). The *BCHE*A* allele shows frequencies ranging from 1.5–7.3% in European populations (Kalow, 1992), but is rarely found or is absent in the majority of Amerindian populations studied (Arends et al., 1967; Tashian et al., 1967; Vergnes and Quilice, 1970; Vergnes et al., 1976; Goedde et al., 1977; Primo-Parmo et al., 1986; Guerreiro et al., 1987; Alcântara et al., 1995). However, appreciable frequencies were reported in some Amerindian groups: a frequency of 3.1% for *BCHE*A* among Makiritare Indians from Venezuela was detected by Arends et al. (1970), a 3.8% frequency was found in a sample of the Mayan population from Mexico (Vergnes and Quilice, 1970), and a 1.6% frequency was revealed in Atacameño Indians from the oasis of Toconao, northern Chile (Goedde et al., 1984). In contrast to the well-studied geographical distribution of the *BCHE*A* variant, fewer population data are available for the *BCHE*F* allele. This may be in part due to difficulties in the analytical technique for identifying this variant. An enzymatic inhibition test found *BCHE*F* allele frequencies from 0.12–1.09% in Europeans (Boman and Habib, 1983), 12.9% in India, and 1.09% for Egyptians (Singh et al., 1971; Boman and Habib, 1983). This allele has not been found in most Native Ameri-

*Correspondence to: Mónica P. Acuña, Programa de Genética ICBM, Facultad de Medicina, Universidad de Chile, Casilla 70061, Santiago 7, Chile. E-mail: macuna@machi.med.uchile.cl

Received 5 October 2001; accepted 1 November 2002.

DOI 10.1002/ajpa.10222

can populations (Tashian et al., 1967; Arends et al., 1967; Alcântara et al., 1995). However, data from Alcântara et al. (1995) show a high frequency of the *BCHE*F* allele in Urubu-Kaapor (7.1%) and Asurini (2.9%) Brazilian Indians.

Most of genotypes with the *BCHE*K* allele, which codes for a quantitative variant, cannot be detected by the enzymatic inhibition test; it can be identified by this method only in heterozygotes for the allele *BCHE*A* (Bartels et al., 1992). However, the *BCHE*K* variant can be clearly distinguished from the other alleles by direct DNA analysis (Shibuta et al., 1994). There are a few studies based on direct DNA analysis that describe the allelic frequencies of *BCHE*K* in different populations. They show similar frequencies of the *BCHE*K* variant in populations of different ethnic and geographic origins (Souza et al., 1998).

The objective of this paper was to estimate the allelic frequencies of *BCHE*U*, *BCHE*A*, *BCHE*F*, and *BCHE*K* in Mapuche aborigines of the VIII and X regions of southern Chile, and to compare these frequencies with those of other Amerindian populations, to test the hypothesis that *BCHE*A*, *BCHE*K*, and *BCHE*F* were not present in native Amerindian populations. We shall demostrate the limitations of the enzymatic method, especially in the detection of the *BCHE*F* allele.

MATERIALS AND METHODS

We studied a sample of 271 Mapuches, of whom 33.2% are Huilliches from San Juan de la Costa, 41.7% are Cuncos from the region of Carelmapu and of Chiloé island, and 25.1% are Pehuenches from Trapa-Trapa. The Mapuche Indians studied may be briefly described as follows: 1) Huilliches or "people of the south" are found between the Toltén River and the Gulf of Reloncaví (Latcham, 1928). 2) Cuncos, meaning "raceme" or "bunch," are found between the Rio Bueno and Chiloé island, though some authors prefer to use the term Cuncos only for the indigenous population that inhabits Chiloé island (Latcham, 1928; Harb et al., 1998). The Cuncos and Huilliches have had a long history of contact with non-Indians, and their way of life is similar to that of rural Chileans. 3) Pehuenches, or "people of the *Araucaria* trees," live in "Alto Bio-Bio" (upper Bio-Bio River valley and surroundings), in the VIII and IX regions of Chile. Until 1980, the area named Trapa-Trapa (37°43′S, 7°16′W), which corresponds to a border valley at the foot of the Copahue volcano, near the Argentinean area of the same name, could only be reached after a long trek on horseback or by helicopter. This inaccessibility contributed to maintaining the isolation of this group; they have thus retained their cultural and biological identity (Latcham, 1928; Aspillaga et al., 1988; Llop et al., 1993).

From each informed volunteer, we collected a blood sample in EDTA for DNA preparation, as well as a serum sample for butyrylcholinesterase activity measurement and phenotyping. The BChE phenotypes were determined by assaying BChE enzyme activity with alpha-naphthylacetate as substrate, in the presence or absence of either dimethyl carbamate of (2-hydroxy-5 phenylbenzyl)-trimethyl ammonium bromide (RO2-0683) or DL-propranolol as inhibitors. Fast Red TR salt was used as color reagent, and sodium lauryl sulfate as an inhibitor to stop the enzyme reaction (Morrow and Motulsky, 1968; Whittaker et al., 1981; Alcântara, 1989). A Shimadzu spectrophotometer at 540 nm was used for quantification. The phenotypic characterization is expressed as the percentages of inhibition of the enzyme activity caused by DL-propranolol or by RO2-0683, which are called PN and RON numbers, respectively. They allow the "usual" phenotype to be distinguished from most of the "unusual" ones (BCHE UA, BCHE AK, BCHE AF, and BCHE A) (Alcântara et al., 1991a,b; Picheth et al., 1994). The increasing number of possible genotypes has complicated identification of the different genotypes by biochemical analyses, including the inhibition test; however, current molecular biology techniques (PCR-PIRA, primer-introduced restriction analysis, Shibuta et al., 1994) permit detection of the *BCHE*K* variant. To determine the *BCHE*K* variant type, a subsample of 52 Huilliche individuals was used. DNA was isolated by routine methods and amplified by PCR (approximately 100 ng) in 25 μl total volume, which included PCR buffer, dNTP, 50 pmol/l of each primer, and 1 unit of Taq DNA polymerase (Promega). Two oligonucleotide primers were synthesized specifically to amplify part of *BCHE* exon 4: primer 1 (3′ primer: 5′-CCTGCTTTC CACTCCCATGCTG-3′) and primer 2 (5′ primer: 5′-CGAAATTATTTTTCAGTTAATGAAACAGATA-AAAATTT-3′). Amplification of DNA fragments (106 bp) was performed with the following sequence: 35 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, and a further extension at 72°C for 10 min. The PCR products (10 μl) were digested with 1 U of enzyme Fun4HI (New England Biolabs) at 37°C overnight. DNA fragments were separated in agarose gel (3%) containing ethidium bromide with a DNA molecular weight marker (100 bp, New England Biolabs) for comparison (Shibuta et al., 1994; Jensen et al., 1996).

To resolve doubts about the veracity of determinations involving the allele *BCHE*F*, we typed individuals who were classified by enzymatic techniques as BCHE UF for two different point mutations associated with the fluoride-resistant phenotype. Fluoride-1 and fluoride-2 have a nucleotide substitution which changes Thr 243 to Met (A<u>C</u>G to A<u>T</u>G) and Gly 390 to Val (G<u>G</u>T to G<u>T</u>T), respectively (Nogueira et al., 1992).

In order to estimate the allelic frequencies of *BCHE* by direct count, we assumed that the techniques utilized discriminate genotypes. To obtain a consistent (if biased) estimation of allele frequencies, we ignored the *BCHE*K* allele in the one

TABLE 1. Phenotype frequencies, allele frequencies, standard errors of allele frequencies for BChE *and Spanish component in Mapuche tribes*

Phenotype	N	Phenotype frequencies (%)	Allele	Allele frequencies (%)	Standard error (%)	Spanish component (%)
Pehuenches						
BCHE U	62	91.18	*BCHE*U*	95.59	1.76	10.3
BCHE UF	6	8.82	*"BCHE*F"*	4.41	1.76	
Total	68	100.00		100.00		
Huilliches						
BCHE U	82	91.11	*BCHE*U*	95.00	1.62	22.1
BCHE UF	7	7.78	*"BCHE*F"*	3.89	1.44	
BCHE A	1	1.11	*BCHE*A*	1.11	0.78	
Total	90	100.00		100.00		
Cuncos						
BCHE U	98	86.73	*BCHE*U*	93.33	1.66	25.9
BCHE UF	13	11.50	*"BCHE*F"*	5.78	1.55	
BCHE UA	1	0.88	*BCHE*A*	0.89	0.62	
BCHE AK	1	0.88				
Total	113	100.00		100.00		
Total						
BCHE U	242		*BCHE*U*	94.45	0.98	20.16
BCHE UF	26		*"BCHE*F"*	4.81	0.92	
BCHE UA	1		*BCHE*A*	0.74	0.37	
BCHE AK	1					
BCHE A	1					
Total	271			100.00		

TABLE 2. Frequencies of BChE*K *allele in different populations*

Population	No. of alleles	*BChE*K* frequency (% ± SE)	Method	Reference
North America	94	(12.8 ± 3.4)	DNA sequencing	Bartels et al. (1992)
Scotland	102	(19.6 ± 3.9)	PCR ARMS	Gaffney and Campbell (1994)
Japan	232	(16.4 ± 2.4)	PCR PIRA	Shibuta et al. (1994)
Japan	280	(17.5 ± 2.3)	PCR PIRA	Izumi et al. (1994)
Denmark	50	(18.0 ± 5.4)	PCR PIRA	Jensen et al. (1996)
Brazil				Souza et al. (1998)
Whites	190	(18.4 ± 2.8)	PCR PIRA	
Admixed	164	(17.1 ± 2.9)	PCR PIRA	
Chile				
Huilliches	104	(5.76 ± 2.3)	PCR PIRA	Present study

BCHE AK individual found, treating this individual as if he were haploid *BCHE*A*. Spanish mixture was estimated according to the method of Bernstein (1931), using the *ABO* and *Rh* loci with the assumption that indigenous populations of South America did not have alleles *ABO*A* and *ABO*B* or *cde* (Salzano and Callegari, 1988; Rothhammer and Llop, 2003).

RESULTS

Table 1 presents the estimates of phenotype and allele frequencies, along with their standard errors, for the different BChE variants using the enzymatic methodology, and the Spanish mix percentages for each group, an estimation based on alleles *ABO*O* and *ABO*A* of the ABO system, and on the *cde* haplotype of the independent Rh system. The *BCHE*A* allele was found in Huilliches and Cuncos, while the *BCHE*K* allele only appeared in Cuncos. Since the enzymatic methodology does not detect BCHE UK phenotypes, the frequency of *BCHE*U* is overestimated when allele *BCHE*K* is present in a population. The "*BCHE*F*" allele appeared in all three tribes, and the allelic frequency was not significantly different among them ($\chi^2 = 0.82$; $P = 0.66$). The percentages of enzymatic inhibition between the individuals BCHE UA (RON, 62.0%; PN, 50.5%), BCHE AK (RON, 47.3%; PN, 35.0%), and BCHE A (RON, 12.40%; PN, 1.8%) were categorically different. On the other hand, average percentage of enzymatic inhibition from individuals with phenotypes BCHE U (RON, 88.58%; PN, 72.32%) and BCHE UF (RON, 84.18%; PN, 67.13%) were not significantly different ($P > 0.05$).

It is worth noting that the Pehuenches, who show the lowest percentage of Spanish admixture, did not have the *BCHE*A* variant. In contrast, one Huilliche individual was apparently homozygous *BCHE*A/*A*; however, this person had European surnames and features.

Table 2 compares the allele frequencies of *BCHE*K* in Huilliches (PCR-PIRA) to those of previously published studies in other populations. Of the total 52 Huilliches individuals, 46 presented *BCHE *U/*U* (88.46%), U being the "usual" allele, and six were heterozygotes *BCHE*U/*K* (11.54%). Average percentages of enzymatic inhibition between individuals *BCHE*U/*K* (PN, 72.09%; RON,

87.8%) and *BCHE *U/*U* (PN, 71.09%; RON, 87.7%) were not significantly different. The *BCHE*K* observed frequency in Huilliches was significantly lower ($P \leq 0.05$; Z test) than all seven frequencies reported by the researchers listed in Table 2. Comparison of results obtained from the PCR-PIRA and enzymatic inhibition techniques showed that the latter is not a good method for discriminating the BCHE UK phenotype.

Using the Hardy-Weinberg equilibrium formula (Hartl and Clark, 1989), we calculated the expected number of the three genotypes from the total allele frequencies: they were 46.17, 5.65, and 0.17, respectively, for genotypes *BCHE*U/*U*, *BCHE*U/*K*, and *BCHE*K/*K*, which coincided with the numbers observed for each genotype.

The enzymatic techniques identified 26 individuals as carrying the allele "*BCHE*F*." We typed 20 of these individuals for the two point mutations associated with this variant (Nogueira et al., 1992). None of these showed either of the two mutations.

DISCUSSION

A large number of population genetic studies of South American Indians have shown that the genetic variability present in the majority of studied loci is less than that found in non-Amerindian populations; the allele frequencies at these loci have also proven to be quite homogeneous. This makes it likely that all these tribes derive from one major parental stock that entered the South American continent in one or very few places (Salzano and Callegari, 1988). The *BCHE* locus is no exception to this rule. The majority of studies of South American Indian populations have not found unusual variants of this locus; their presence in some groups may be explained by gene flow from non-Amerindian populations (Salzano and Callegari, 1988).

Arends et al. (1967) suggested that the *BCHE*A* allele should be rare in South American Indians. The absence of *BCHE*A* in Pehuenches is in accordance with this idea, and emphasizes the rarity of this atypical allele in Amerindians. The hypothesis of Arends et al. (1967) is reinforced by indications of European ancestry (e.g., surnames, physical features; data not shown) in the few individuals that presented the allele *BCHE*A* in this study.

Using enzymatic methods, the frequencies of "*BCHE*F*" estimated from our samples are higher than those reported for European populations (Whittaker, 1980; Kalow, 1992), and notably higher than those of various South American populations, in which the "*BCHE*F*" allele is rare or absent (Alcântara et al., 1991b), in contrast to the frequency reported for the indigenous population Urubu-Kaapor by Alcântara et al. (1995). These results are probably due to an overestimation of the frequency of allele "*BCHE*F*," since the standard enzymatic analysis does not differentiate categorically between the phenotypes BCHE U and BCHE UF. These results led us to believe that allele *BCHE*F* is very infrequent in the Mapuche population, which was reinforced by the fact that one individual was found with phenotype BCHE AK, but none were found with BCHE AF using this methodology. The results of examining individuals supposedly heterozygous for the *BCHE*F* allele for point mutations were striking. Neither of these two mutations was found in any of the 20 individuals tested, which corroborates that the enzymatic analysis technique is not adequate for discriminating BCHE UF phenotypes. Given the limitations of the enzymatic method, we suggest that DNA technology should be used for population studies in the future.

The presence of the *BCHE*K* allele in the Huilliches is best explained by gene flow from European populations. We thus would expect to estimate the same frequency of admixture for this allele as for the ABO and Rh systems. To test this, we must suppose that 1) the European population that mixed with the indigenous group had a *BCHE*K* allele frequency similar to those found in current European groups (Souza et al., 1998), 2) originally, the indigenous Chilean population did not have the *BCHE*K* alleles, and 3) the estimations of admixture are not too far from the real values. For the Huilliches sample, using the formula of Bernstein (1931) and 22.1% as the estimate of degree of European admixture (Table 1), we found an expected frequency of 4.2% for the *BCHE*K* allele. This agrees very well with the observed frequency (5.76%), from which we conclude that the difference in frequency of *BCHE*K* in Huilliches and the other sampled populations is due to different degrees of European admixture. Even if the degrees of admixture estimated by ABO and Rh frequencies are not too precise, the concordance of their values with of BCHE lends strength to our argument.

Which factors may have been important in producing current observed frequencies? Genetic drift most probably played a role in the past in these populations, as has been suggested for many Amerindian groups (Rothhammer and Llop, 2003). However, since the arrival of Europeans, their isolation has diminished: all Chilean groups that have been examined show significant admixtures (Rothhammer and Llop, 2003). Since observed frequencies of *BCHE* alleles agree with the estimated degree of admixture for these tribes, there is no evidence that genetic drift played an important role in determining the frequencies allele of these Mapuches populations.

Souza et al. (1998) suggested that natural selection acted on the *BCHE* locus, based on the low frequency of silent alleles and the similarity of *BCHE*K* allele frequencies among different groups (Table 2). These authors did not specify that they meant stabilizing selection, but this is the kind of selection that would maintain similar allele frequencies. Although our results cannot exclude the possibility of stabilizing selection at the locus, finding a frequency of *BCHE*K* significantly different from

those found in populations of different ethnic and geographic origins casts doubt on the validity of this hypothesis for this allele.

ACKNOWLEDGMENTS

We thank José Saa, Héctor Pizarro, Cristian Urrea, and Donisia Sepúlveda for their efficient laboratory assistance.

LITERATURE CITED

Acuña M, Llop E, Rothhammer F. 1994. Composición genética de la población chilena: Los Atacameños de la comuna de San Pedro de Atacama. Rev Med Chile 122:1126–1133.

Alcântara VM. 1989. Determinaçao dos fenótipos CHE1 UF e CHE1 AK em amostras de brancos e negróides de Curitiba. M.Sc. thesis, Universidade Federal do Paraná, Curitiba.

Alcântara VM, Chautard-Freire-Maia EA, Picheth G, Vieira MM. 1991a. A method for serum cholinesterase phenotyping with a clear discrimination of the CHE1 UF heterozygotes. Rev Bras Genet 14:841.

Alcântara VM, Chautard-Freire-Maia EA, Culpi L.1991b. CHE1 UF serum cholinesterase phenotype in whites and non-whites from southern Brazil as determined by a new method. Hum Hered 41:103–106.

Alcântara VM, De Lourenco MAC, Salzano FM, Petzl-Erler ML, Coimbra JR, Santos RV, Chautard-Freire-Maia EA.1995. Butyrylcholinesterase polymorphisms (BCHE and CHE2 loci) in Brazilian Indian admixed populations. Hum Biol 67:717–726.

Arends T, Davies D, Lehmann H. 1967. Absence of variants of usual serum pseudocholinesterase (acylcholine acylhydrolase) in South American Indians. Acta Genet (Basel) 17:13–16.

Arends T, Weitkamp LR, Gallango ML, Neel JV, Schultz J. 1970. Gene frequencies and microdifferentiation among the Makiritare Indians. II. Seven serum protein systems. Am J Hum Genet 22:526–532.

Aspillaga E, Paredes C, Kalwasser J. 1988. Los sistemas ABO y Rh en la población de Trapa-Trapa, comuna de Santa Bárbara, VIII región. Rev Chil Antropol 7:115–121.

Bartels CF, Jensen FS, Lockridge O, van der Spek AFL, Rubinstein HM, Lubrano T, La Du BN. 1992. DNA mutation associated with the human butyrylcholinesterase K variant and its linkage to the atypical variant mutation and other polymorphic sites. Am J Hum Genet 50:1086–1103.

Bernstein F. 1931. Die geographische Verteilung der Blutgruppen und ihre antrhopologische Bedeutung. In: Comitato Italiano per lo studio del problemi della populazione. Rome: Instituto Poligrafico dello Stato. p 227–243.

Boman H, Habib Z. 1983. Serum cholinesterase loci E1 and E2 polymorphisms among Egyptians. Hereditas 99:1–6.

Gaffney D, Campbell RA. 1994. A PCR method to determine the Kalow allele frequency and its significance in the normal population. J Med Genet 31:248–250.

Goedde HW, Benkmann HG, Agarwal DP, Kroeger A. 1977. Genetic studies in Ecuador: acetylador phenotypes, red cell enzyme, and serum protein polymorphisms of Shuara Indians. Am J Phys Anthropol 47:419–425.

Goedde HW, Rothhammer F, Beckman HG, Bogdansky P. 1984. Ecogenetic studies in Atacameño Indians. Hum Genet 67:343–346.

Guerreiro JF, dos Santos SEB, de Lourenço C, Primo-Parmo SL, Chautard-Freire-Maia EA. 1987. Serum cholinesterase polymorphism (CHE1 and CHE2 loci) in Indians from the Amazon region of Brazil: Urubu-Kaapor and Assurini tribes. Rev Bras Genet 4:781–785.

Harb Z, Llop E, Moreno R, Quiroz L. 1998. Poblaciones costeras de Chile: marcadores genéticos en cuatro localidades. Rev Med Chile 126:753–760.

Hartl D, Clark A. 1989. Principles of population genetics. Sunderland, MA: Sinauer Associates, Inc.

Izumi M, Maekawa M, Kanno T. 1994. Butyrylcholinesterase K variant in Japan: frequency of allele and associated enzyme activity in serum. Clin Chem 40:1606–1607.

Jensen FS, Nielsen LR, Schwarts M. 1996. Detection of plasma cholinesterase K variant by PCR using an amplification-created restriction site. Hum Hered 46:26–31.

Kalow W. 1992. Pharmacogenetics of drug metabolism. New York: Pergamon Press.

Latcham R. 1928. La prehistoria Chilena. Santiago, Chile: Soc Imp y Lit Universo.

Llop E. 1996. Genetic composition of Chilean aboriginal populations: HLA and other genetic marker variations. Am J Phys Anthropol 101:325–332.

Llop E, Harb Z, Acuña M, Moreno R, Barton S, Aspillaga E, Rothhammer F. 1993. Composición genética de la población chilena: los pehuenches de Trapa-Trapa. Rev Med Chile 121: 494–498.

Matson GA, Sutton HE, Etcheverry R, Swarson J, Robinson A. 1967. Distribution of hereditary blood groups among Indians in South America. Am J Phys Anthropol 27:157.

Morrow AC, Motulsky AG. 1968. Rapid screening method for the common atypical pseudocholinesterase variant. J Lab Clin Med 71:350–356.

Nogueira CP, Bartels CF, McGuire MC, Adkins S, Lubrano T, Rubenstein HM, Lightstono H, Van Der Spek AFL, Lockridge O, La Du BN. 1992. Identification of two different point mutations associated with the fluoride-resistant phenotype for human butyrylcholinesterase. Am J Hum Genet 51:821–828.

Picheth G, Fadel-Picheth C, Primo-Parmo SL, Chautard-Freire-Maia EA, Viera MM. 1994. An improved method for butyrylcholinesterase phenotyping. Biochem Genet 32:83–89.

Primo-Parmo SL, Chautard-Freire-Maia EA, de Lourenço MAC, Salzano FM, de Melo e Freitas MJ. 1986. Studies on serum cholinesterase (CHE1 and CHE2) in Brazilian Indian and admixed populations. Rev Bras Genet 3:467–478.

Rothhammer F, Llop E. 2003. Antropología biológica de Chile. Santiago, Chile: Amphora.

Salzano FM, Callegari-Jacques SM. 1988. South American Indians: a case study in evolution. Oxford: Clarendon Press.

Shibuta K, Abe M, Suzuki T. 1994. A new detection method for the K variant of butyrylcholinesterase based on PCR primer introduced restriction analysis (PCR-PIRA). J Med Genet 31: 576–579.

Singh S, Amma MKP, Sareen KN, Goedde HW. 1971. A study of the pseudocholinesterase polymorphism among a Panjabi population. Hum Hered 21:388–393.

Souza RLR, Castro RMV, Pereira L, Freund AA, Culpi L, Chautard-Freire-Maia EA. 1998. Frequencies of the butyrylcholinesterase K mutation in Brazilian populations of European and African origin. Hum Biol 70:965–970.

Tashian RE, Brewer GJ, Lehmann H, Davies DA, Rucknagel DL. 1967. Futher studies on the Xavante Indians. V. Genetic variability in some serum and erythrocyte enzymes, hemoglobin and the urinary excretion of β-amino-isobutyric acid. Am J Hum Genet 19:524–531.

Vergnes H, Quilice JC. 1970. Le gène E^a_1 de la pseudocholinestérase sérique (A.C.A.H.) chez les Amérindiens. Ann Genet 13: 96–99.

Vergnes H, Quilice JC, Gherardi M, Bejarano G. 1976. Serum and red cell enzyme variants in an American tribe, the Sirionós (eastern Bolivia). Hum Hered 26:252–262.

Whittaker M. 1980. Plasma cholinesterase variants and anaesthetist. Anaesthesia 35:174–197.

Whittaker M, Britten JJ, Wicks RJ. 1981. Inhibition of the plasma cholinesterase variants by propranolol. Br J Anaesth 53:511–516.