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The Cloned Butyrylcholinesterase (*BCHE*) Gene Maps to a Single Chromosome Site, 3q26

P. W. ALLDERDICE,* H. A. R. GARDNER,* D. GALUTIRA,* O. LOCKRIDGE,†
B. N. LADU,‡ AND P. J. MCALPINE§

*Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3V6, Canada; †Eppley Institute, University of Nebraska Medical Center, Omaha, Nebraska 68198-6805; ‡Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0626; and §Department of Human Genetics, University of Manitoba, Winnipeg, Manitoba R3E OW3, Canada

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Human tissues have two distinct cholinesterase activities: acetylcholinesterase and butyrylcholinesterase. Acetylcholinesterase functions in the transmission of nerve impulses, whereas the physiological function of butyrylcholinesterase remains unknown. An atypical form of butyrylcholinesterase or the absence of its activity leads to prolonged apnea following administration of the muscle relaxant suxamethonium. Inheritance of these butyrylcholinesterase variants is consistent with the enzyme activity being encoded in a single autosomal locus, *BCHE* (formerly *CHE1* and E_1), which has been assigned to chromosome 3. Previous *in situ* hybridization of a *BCHE* cDNA probe gave evidence of homologous sequences at 3q26 and 16q11–q23, raising the possibility of more than one locus coding for butyrylcholinesterase [H. Soreq, R. Zamir, D. Zevin-Sonkin, and H. Zakut (1987) *Hum. Genet.* 77: 325–328]. Using a different cDNA probe hybridized *in situ* to 46,XX,inv(3)(p25q21) metaphase chromosomes, we report here the localization of *BCHE* to a single autosomal location: 3q26.

INTRODUCTION

Humans have two major types of cholinesterases: acetylcholinesterase (AChE,[1] EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8), which differ in their tissue distribution and catalytic properties (reviewed by Massoulié and Bon, 1982; Massoulié and Toutant, 1988; Chatonnet and Lockridge, 1989). AChE, also referred to as "true cholinesterase," hydrolyzes acetylcholine at cholinergic synapses, whereas the true biological function of BChE remains obscure. BChE is known to hydrolyze the muscle relaxant suxamethonium, which is commonly administered during anesthesia. The production of prolonged apnea in about 1 in 2000 members of the general population following the administration of suxamethonium has been attributed to abnormal genetic variation of BChE (Kalow and Gunn, 1957). Sensitivity to suxamethonium may be due to the atypical form of BChE, which has reduced affinity for this substrate (reviewed by Whittaker, 1986) as the result of a point mutation in the *BCHE* structural gene (McGuire *et al.*, 1989), or to the virtual absence of BChE activity. This latter phenotype, referred to as "silent," is attributed to a number of different mutations in the *BCHE* gene (Nogueira *et al.*, 1990). Additional variants of the *BCHE* gene occurring as restriction fragment length polymorphisms have now been reported (Bartels *et al.*, 1990; McAlpine *et al.*, 1991).

The *BCHE* locus, initially shown to be linked to the transferrin (*TF*) locus (Robson *et al.*, 1966), was subsequently mapped to chromosome 3 (Sparkes *et al.*, 1984; Yang *et al.*, 1984) and shown to be distal to 3q21 (McAlpine *et al.*, 1987). When the first full-length cDNA clone of the *BCHE* gene was used for *in situ* hybridization mapping studies, signals were reported initially at 3q21, 3q26, and 16q11–q23 (Soreq *et al.*, 1987a) and subsequently at 3q26 and 16q11–q23 (Soreq *et al.*, 1987b). The occurrence of a site of hybridization on chromosome 16 prompted speculation that it could represent the locus encoding the C5 isozyme, detected by starch gel electrophoresis as an additional molecular species of BChE in 8% of the Caucasian population (Whittaker, 1986). A recent linkage study has shown that the *CHE2* locus (formerly *E2*), to which the C5 isozyme is attributed, is linked to the γ-crystallin gene cluster (Eiberg *et al.*, 1989), previously assigned to chromosome 2 (Willard *et al.*, 1985; den Dunnen *et al.*, 1985; Shiloh *et al.*, 1986).

Five *BCHE* genomic clones have been isolated (Arpagaus *et al.*, 1990), using cDNA probes encoding the catalytic subunit of tetrameric enzyme (McTiernan *et al.*, 1987). Analysis of these clones indicates that the *BCHE* gene is at least 73 kb long and contains four exons (Arpagaus *et al.*, 1990).

Our recent discovery of an RFLP of the *BCHE* locus (McAlpine *et al.*, 1991) using two of the five independently isolated genomic *BCHE* clones (Arpagaus *et al.*, 1990) prompted us to identify the chromo-

[1] AChE and BChE refer to the enzyme; *BCHE* refers to the gene.

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somal origin of these clones for the facilitation of the interpretation of data from recombinational and physical versions of the map of chromosome 3 in our laboratories.

MATERIALS AND METHODS

Probe

The probe used contained an insert of 2.3 kb of *BCHE* sequence, derived from a full-length cDNA, cloned by Dr. O. Lockridge.

Lymphocyte Cultures

Lymphocytes were isolated from a peripheral blood sample obtained from an inversion heterozygote, who is a member of a very large kindred in which an inv(3)(p25q21) segregates (Allderdice *et al.*, 1975). Following culturing of the lymphocytes as described by Buckle and Craig (1986) for 72 h, 20 μg/ml bromodeoxyuridine (BRdU) was added and 16–17 h later the block was released by the addition of medium containing 10^{-5} *M* thymidine. Cells were harvested 4–5 h later after a 20-min exposure to 0.05 μg/ml colcemid and 13 min in 0.075 *M* potassium chloride. The cell suspension was dropped and air-dried on slides.

In situ hybridization was carried out as described by Craig *et al.* (1988). Slides were treated with RNase (100 μg/ml in 2× SSC) at 37°C for 1 h, rinsed in 2× SSC, dehydrated through alcohol series, and air-dried. The slides were denatured at 65°C for 4 min in 70% formamide and 0.1 m*M* EDTA in 2× SSC, rinsed twice in 2× SSC (the first chilled to 4°C), and then dehydrated. The *BCHE* probe (50 ng) was radiolabeled to a specific activity of 6.1×10^7 dpm/μg with [^{3}H]dCTP by random primer extension using the multiprime DNA labeling system (Amersham) according to the manufacturer's specifications. The labeled probe was added at a concentration of 125 ng/ml to 350 μl hybridization mix (Sigma) and denatured by boiling for 5 min. Then 30 μl of the labeled probe was placed on each slide, the coverslip was sealed with rubber cement, and the slides were incubated at 43°C for 20 h. The slides were rinsed in 5× SSC, washed in 2× SSC three times for 20 min each, and split into two batches, one being washed in 0.2× SSC at 60°C and the other in 2× SSC at 65°C. Both washes were for 1 h (with one change) after which all slides were washed two times in 0.2× SSC for 30 min and two times in 0.1× SSC for 30 min and dehydrated.

Slides were then dipped in Kodak NTB2 emulsion and exposed 13–18 days at 4°C. Following development in Kodak D19 for 7 min at 20°C, slides were fixed, washed, then stained with Hoechst 33258 (10 μg/ml in 2× SSC) for 30 min, exposed in 2× SSC to uv light for 1 h, and stained with 10% Giemsa for 30 min. Grains on, or touching, the chromatids were scored according to chromosomal location, with clusters of grains counted as one grain.

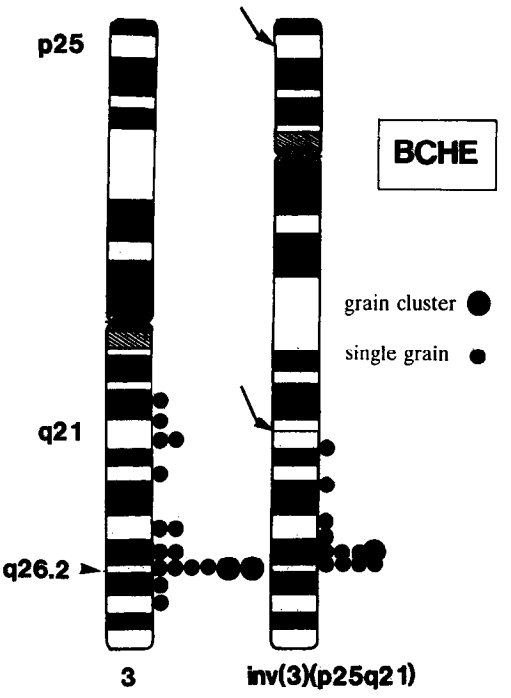

FIG. 1. Idiogram of human chromosomes from an inv(3)(p25q21) heterozygote showing the distribution of silver grains after autoradiography of ^{3}H-labeled *BCHE* cDNA probe.

RESULTS

A clear signal was present on both the normal chromosome 3 and the inv(3) chromosome at 3q26 (Fig. 1) on the slides washed at the higher stringency (2× SSC). Silver grains on the other autosomes, including chromosome 16, appeared to be distributed randomly, without evidence of clustering. Of the total 131 grains scored from 53 complete 46,XX,inv(3) metaphases, 10.9% mapped distal to the 3q21 breakpoint. When only chromosome 3 and the inv(3) were examined, an additional 10 grains were located in 6 cells. Combined counts from partial and complete cells showed 28 of 32 grains on chromosome 3 and/or the inv(3) distal to the q21 breakpoint. Fifty percent of the grains distal to this breakpoint, including 3 clusters, mapped to 3q26. The signals on the normal and inv(3) chromosome were in comparable locations and not influenced by the inv(3)(p25q21), indicating that the *BCHE* locus is clearly distal to 3q21, which contains the *TF* locus to which it is linked.

DISCUSSION

The clustering of signal at 3q26 on both the normal and the inv(3) chromosomes indicates a single geno-

mic site for *BCHE* coding sequences. A single copy of the *BCHE* locus, as indicated by the data presented here, is consistent with the data obtained from the nucleotide sequence and polymerase chain reaction studies reported earlier by Arpagaus *et al.* (1990) and La Du *et al.* (1991). The absence of signal on 2q, where *CHE2* has been assigned, suggests that the locus to which the C5+ phenotype is attributed does not code for a butyrylcholinesterase. Furthermore, no evidence of a signal with the *BCHE* cDNA probe to any location on chromosome 16 was obtained. Thus, the previous report of Soreq *et al.* (1987b) of a *BCHE*-like sequence on chromosome 16 detected by *in situ* hybridization with a different cDNA probe cannot be confirmed. The finding of a single genomic location for the gene encoding butyrylcholinesterase gives confidence for using RFLP analysis of this locus for linkage studies of chromosome 3 markers.

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Note added in proof. Since this manuscript was submitted, G. Gaughan *et al.* (1991, *Genomics* **11:** 455–458) have confirmed the regional localization of *BCHE* to 3q26 using a PCR-derived probe.

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