Genomic and Transcriptional Characterization of the Human *ACHE* Locus: Complex Involvement with Acquired and Inherited Diseases

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Abstract

Background: Abnormal levels of the acetylcholinesterase enzyme or aberrations involving the long arm of chromosome 7, harboring the *ACHE* gene at 7q22, occur in various diseases such as Alzheimer's, Parkinson's, and leukemias. However, the cause(s) of these abnormalities are still unknown.

Objective: To search for the genomic elements and transcriptional processes controlling *ACHE* gene expression and the plausible stability of its locus, by isolating, sequencing and characterizing the human (h)ACHE locus and its mRNA products.

Methods: Three clones containing the *ACHE* gene were isolated from a human chromosome 7 cosmid library. Two of these clones were thereafter sequenced and searched for repetitive elements, open reading frames and corresponding expressed sequence tags. Reverse transcription-polymerase chain reaction was employed to further explore these findings.

Results: The locus harboring the G,C-rich *ACHE* gene was found to be exceptionally rich in Alu repeats. It includes an additional, inversely oriented gene (*ARS*), tentatively associated with arsenite resistance. EST clones corresponding to both genes were found in cDNA libraries from 11 different human tissue sources, with ARS expressed in 10 additional tissues. Co-regulation of brain *ACHE* and *ARS* was suggested from their mutually increased expression following acute psychological stress.

Conclusions: The abundance of Alu retrotransposones may predispose the *ACHE* locus to chromosomal rearrangements. Additionally, coordinated transcriptional regulation is implied from the joint ARS-AChE expression in stress insult responses. Disease-related changes in AChE may therefore reflect locus-specific regulation mechanisms affecting multiple tissues.

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The acetylcholine hydrolysing enzyme acetylcholinesterase (acetylcholine acetylhydrolase, AChE, EC 3.1.1.7) terminates cholinergic neurotransmission and thus controls central and peripheral nervous system functions. AChE abnormalities have been reported in various pathologies, including nervous system diseases such as Alzheimer's or Parkinson disease and myasthenia gravis, or cases of intoxication with chemical warfare agents [1]. AChE abnormalities also occur in diseases involving non-cholinergic tissues and cell types, e.g., nocturnal hemoglobinuria [2]. These diseases may reflect non-catalytic activities of the enzyme, which shares sequence homologies and functional properties with a series of non-enzyme cell-cell interaction proteins [reviewed in 3]. In addition to changes in AChE expression, chromosomal aberrations involving band 7q22, harboring the human (h)*ACHE* locus, are associated with various types of leukemias [4].

AChE inhibitors (anti-AchEs, e.g., commonly used agricultural insecticides [5]), increase the risk for non-Hodkin's lymphomas [6]. Chemicals with anti-AChE capabilities also include chemical warfare agents, prophylactic drugs aimed to protect from exposure to such agents [7], and a wide range of anti-AChE drugs used for treating Alzheimer's disease [8] and myasthenia gravis patients [9]. However, both exposure to anti-AChEs and acute psychological stress induce AChE overproduction [10,11]. Moreover, transgenic mouse models with AChE overproduction presented early cognitive deterioration [12], progressive neuromuscular deformities [13], and typical characteristics of stress insults in their brain (Sternfeld et al., submitted). This suggests long-lasting delayed consequences for AChE accumulation. To evaluate the scope of such consequences, we combined molecular genetic approaches with searches of genomic databases to explore the h*ACHE* locus.

EST = express sequence tags
AchE = acetylcholinesterase enzyme

Materials and Methods

RT-PCR

Primers used for PCR amplification were 5'-CAGG-TCCCGATATTCCACAAT-3' and 5'-GGGCTGACCATA-GGGCATCA-3' for mARS, 5'-TGAAACAACATA-CAATTCCATCATGAAGTGTGAC-3' and 5'-AGG-AGCGATAATCTTGATCTTCATGGTGCT-3' for actin and primers complementary to exon 2 of mAChE, which is common to all AChE isoforms [10]. Samples of PCR products were withdrawn at every third cycle starting at cycle 18 for AChE and actin, and every second cycle starting at cycle 26 for ARS.

Sequence analyses

Searches for open reading frames in the hACHE locus, G,C content as well as repetitive sequences in the *ACHE* upstream sequence and ESTs derived from this locus were performed using the following programs: Grail (http://avalon.epm.ornl.gov/Grail-bin/EmptyGrailForm), Window (the University of Wisconsin GCG software package), Repeat Masker 2 (http://ftp.genome.washington.edu) and Blast (http://www.ncbi.nlm.nih.gov/BLAST/), respectively.

Results

To extend the genomic analysis at the *ACHE* locus, the cloned human *ACHE* gene has been used as a probe for screening a cosmid library [14]. Three cosmid clones were isolated, two of which included a PCR-amplifiable sequence located immediately upstream to the *ACHE* transcription start site [Figure 1]. One of the two cosmid clones containing the *ACHE* upstream region was fully sequenced. and the third clone, not containing this region, was used to extend the sequenced portion downstream to *ACHE*. This yielded a 38 Kb sequence. A search for open reading frames demonstrated two inversely oriented active genes in this locus, *ACHE* and *ARS*, the latter showing high similarity to a rodent gene which is over-expressed in cells exposed to arsenite [15].

Searching the *ACHE* locus for repetitive elements unraveled an extremely high prevalence of Alu repeats, non-viral retrotransposones that are part of the family of short interspersed elements (SINEs) [Figure 2]. Thirty-nine of these repeats were identified along 23 Kb of *ACHE* upstream sequence. In addition, this region was found to be markedly lower in its G,C content than the *ACHE* gene itself.

To determine which tissues express hAChE and hARS, the EST database was searched. ESTs originating from AChE and hARS mRNA transcripts were found in many different tissues, including both adult (brain, intestine, skeletal muscle, colon and kidney) and fetal tissues (liver and spleen, lung and heart) [Figure 3]. hARS mRNA was considerably more abundant; different alternatively spliced hARS mRNA sequences were found in all of the

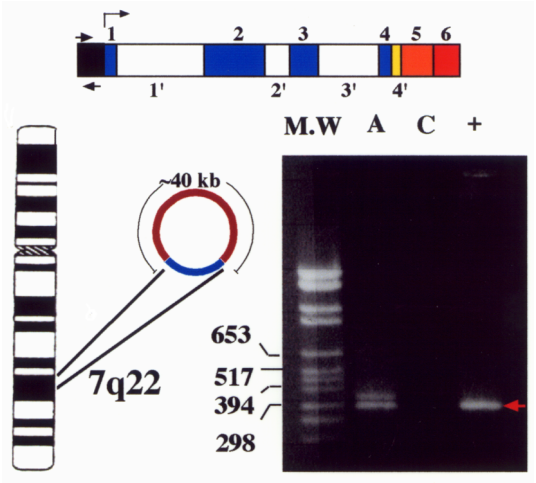

Figure 1. Identification of 7q22 cosmids containing the *ACHE* gene. Shown on top is a schematic description of the PCR identification of cosmids containing the *ACHE* gene from a 7q22 cosmid library and the structure of the *ACHE* gene, with exons represented by filled boxes numbered above and introns represented by open boxes numbered below. Black box represents 600 bp of the proximal promoter with a right-bent arrow designating the transcription start site. Below, the position of the analyzed cosmid on chromosome 7 is drawn and a gel image shows a 294 bp-long PCR product (marked with a red arrow) amplified using primers depicted at the top scheme with thick arrows. This fragment could be amplified from cosmid A (Genebank accession no. Af002993) but not from cosmid C (accession no af237614), both located at 7q22. Plus sign designates a PCR reaction with the plasmid containing *ACHE*'s proximal promoter, serving as template.

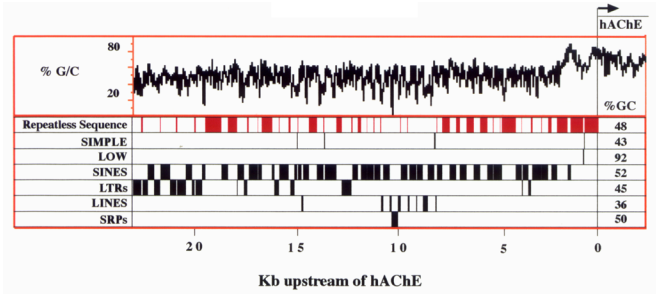

Figure 2. Analysis of the *ACHE* extended promoter. Analysis of the *ACHE* extended promoter (22.5Kb) in terms of G,C content (drawn line), repeatless regions (red boxes), and variable repeat elements, namely simple sequence DNA, low complexity sequence, SINEs, retroviral long terminal repeats (LTRs) and the SRP SINE subtype. Note the abundant SINE sequences, most of which are Alu repeats. Cumulative G,C content (percent) for each of these subgroups is shown (right).

above tissues as well as in B cells, breast tissue, placenta, pituitary gland, fetal lung, cervix, thymus, T cells and fibroblasts. The partial overlap in tissue distribution of mRNA transcripts of hARS and hAChE, together with their close genomic proximity in both primates and rodents (data not shown), raised the possibility of coordinated transcriptional regulation of this entire chromosomal domain. To test whether this is the case, we compared the levels of AChE and ARS in the brain of control mice to those in mice subjected to the forced-swim proto-

RT-PCR = reverse transcription-polymerase chain reaction

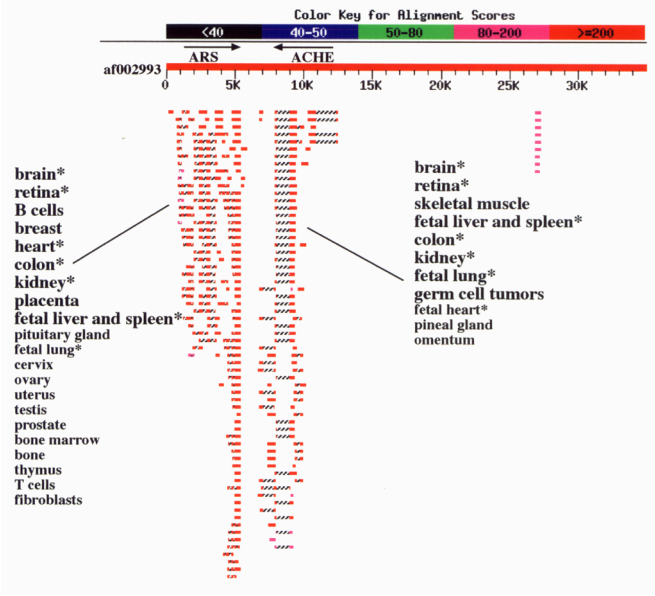

Figure 3. Expression from the 7q22 locus as reflected in the EST database. Shown is the graphical output of the Blast program (http://www.ncbi.nlm.nih.gov/BLAST/) run against the EST database using the af002993 cosmid as a query sequence and restricting the search to human sequences. The identified ESTs are aligned against the corresponding regions of the query sequence, which is shown in red and scaled in bp. ESTs are colored according to their alignment score as depicted in the color key bar (black-hatched portions represent incontinuities in the alignments and therefore indicate introns). Designated are the *ARS* and *ACHE* gene regions and the tissue origins of the different ESTs, with uppercase letters showing highly represented tissues. Asterisks designate tissues expressing both ARS and AChE.

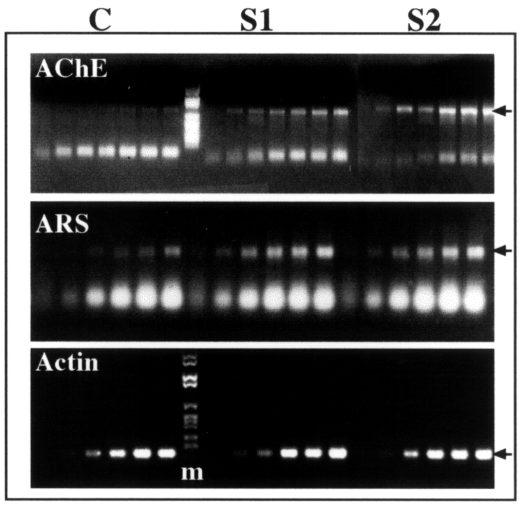

Figure 4. Psychological stress increases both AChE and ARS mRNA levels in the mouse medulla. Shown is one out of three reproducible experiments presenting a semi-quantitative kinetic follow-up of RT-PCR evaluation. Arrow-labeled are AChE, ARS and actin cDNA products in extracts prepared from the brain stem (medulla) region of either a control mouse (C) or two different mice subjected to psychological stress (S) [11]. Densitometry of the fluorescent signals presented eightfold stress-related increases for the AChE transcripts. Fourfold increases were detected for ARS products in brains of stressed as compared to control mice. Actin products showed no difference associated with stress, demonstrating specificity of the AChE and ARS increases. m = size marker.

col causing psychological stress [10]. As expected, stressed mice displayed increased AChE mRNA levels in their brain [11]. In addition, these mice displayed massive co-induction in the brain of the *ARS* gene 2 hours post-stress, while maintaining unchanged actin mRNA levels [Figure 4]. This supports the notion of a stress-associated regulation of gene expression in the *ACHE* locus.

Discussion

Our findings demonstrate ubiquitous expression of the adjacent h*ACHE* and hARS in many different fetal, adult and tumor tissues. This, and the co-induction of mouse (m)AChE and mARS expression under acute psychological stress provide a tentative genomic explanation for the reports of modulated AChE expression in different diseases.

Reported transfection studies mostly referred to the proximal sequence, including up to 300 base pairs of the *ACHE* promoter, as essential for its normal expression [16]. In transgenic mice, the proximal 600 bp of the h*ACHE* promoter failed to induce detectable expression in extra-CNS tissues [12]. Either the transgene insertion site in the host mouse genome or species-specific differences in *ACHE* regulation could be responsible for that. We recently found that transcription factor binding sites located 17 Kbp upstream of the *ACHE* transcription start site regulate osteogenic *ACHE* gene expression [17]. We conclude that similarly to the albumin gene [18] (and several others), exceptionally distal enhancer elements are required for *ACHE*'s proper expression.

The rodent ARS gene (*ARS2)* [15] is over-expressed under arsenite exposure and was therefore tentatively identified as an arsenite-responsive protein. Its human homologue is located downstream to *ACHE*, inversely oriented close to it and co-regulated with it. The sequence of the *ARS* translation product does not resemble any known protein, including the cloned bacterial arsenite resistance protein. As the two genes must have individual promoters, each on a different DNA strand, their co-expression may reflect joint opening of the *ACHE* and *ARS* DNA domains for transcriptional induction. This, in turn, predicts hARS accumulation under all of those conditions that induce AChE overproduction, including acute psychological stress and exposure to AChE inhibitors [11]. One of these inhibitors is arsenite [19], which would induce feedback accumulation of its AChE scavenger, just as it occurs in response to pyridostigmine [10,11]. This suggests that *ARS* accumulates under arsenite exposure only because of co-localization and co-regulation with *ACHE* but is not causally involved in ar-

bp = base pairs
CNS = central nervous system

senite resistance. The *ACHE* locus thus emerges as a jointly controlled domain, perhaps similar to the b-globin locus [20].

AChE accumulation under various stress insults may contribute, through its non-catalytic properties, towards the variable symptoms in Gulf War veterans [21], in patients exposed to agricultural insecticides [5], and in anti-AChE treated Alzheimer's disease patients [22]. Its ubiquitous expression may further predict involvement in many more tissues than the nervous system. Moreover, the remarkable abundance of SINEs, in particular Alu repeats, in the *ACHE* locus implies exceptional susceptibility for tranposition events, which are assisted by the existence of chromosomal breakages [23]. Additionally, Alu repeats may also facilitate unequal crossing-over [24], altogether contributing to instability of this region. Chromosomal rearrangements could result in the loss of upstream transcription factor binding sites, and thus affect *ACHE* gene expression and its capacity to accumulate under stress or exposure to anti-AChEs. This explains the reported chromosomal aberrations involving 7q22 in leukemic patients [25]. The increased risk for non-Hodgkin's lymphoma in farmers exposed to anti-cholinesterases [6] may hence be relevant both to inhibitor-induced accumulation of the AChE protein and to abnormal replication of this locus in lymphocytes. Genomic and transcriptional analysis thus points at the *ACHE* locus as an intersection involved in many pathways, several of which may lead to disease.

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