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Rapid report

Does the AD7c-NTP locus encode a protein?

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Abstract

AD7c-NTP, the only known protein entirely encoded by tandem and nested cassettes of Alu repetitive elements, is reportedly overexpressed in brains of Alzheimer's disease patients [de la Monte et al., J. Clin. Invest. 15 (1997)]. Based on these findings a commercial diagnostic assay ("7c Gold"/"AlzheimAlert") has been developed. We analyzed the published cDNA sequence and compared it to corresponding EST clones as well as the genomic sequences of human and chimpanzee. We come to the conclusion that the existence of the gene and in particular the predicted protein is inconsistent with EST and genomic data. Previously published data need to be reassessed.

Keywords: Alzheimer's disease; Neuronal thread protein; Alu element; Exaptation

Anonymous sequences or those derived from retroposons are frequently recruited or exapted as novel parts of genes [1]. This is particularly apparent in the exonization of Alu elements, where in humans ~4–5% of alternatively spliced mRNAs feature parts of Alu elements in the open reading frame [2,3]. To the best of our knowledge, exaptation of an entire gene from "junk" DNA has not been reported thus far, apart from the study that is being addressed in this communication [4]. The gene is supposed to contain a single exon with an open reading frame (ORF) predicting a 41 kD protein. The locus on chromosome 1 (p36.11) harbours numerous SINE and LINE elements; the reported ORF spans several tandem, even nested Alu elements of different subfamilies. Antibodies against the recombinant protein had been raised. The protein has been detected in neurons with higher expression levels in brains from Alzheimer patients as compared to control individuals.

This would be an exciting finding, but unfortunately, when we compared the reported cDNA sequence to the human genomic sequence [5], we detected numerous mismatches and small indels resulting in frameshifts and premature stop codons predicting an ORF that terminates after 98 instead of 375 amino acids (Fig. 1). In the orthologous chimpanzee locus [chr1:24155382–24156812; http://genome.ucsc.edu/cgi-bin/hgBlat], the ORF also terminates after 98 codons. Within this shorter ORF we count 8 mismatches between human and chimpanzee (5 are synonymous and 3 alter the encoded amino acids). Over the short ORF, the cDNA reported [4] differs by about 11 mismatches and small indels from the genomic sequences, interestingly, in positions where the genomic sequences are identical between human and chimpanzee (Fig. 1). Three ESTs overlap the short ORF (CA437412, BM996475, BI492776) and two (BX110762, AI702327) correspond to the distal part of the presumed 3′ UTR of the longer "ORF" predicted by [4]. The ESTs are identical to the genomic sequence and hence show the same mismatches in comparison with the presumed AD7c-NTP mRNA. The combined evidence makes it highly unlikely that the six reported cDNAs from Alzheimer patients are derived from an additional allele that may be responsible for the development of the neurodegenerative disorder. Nevertheless, this remote possibility is testable by nested RT-PCR and genomic PCR: A small

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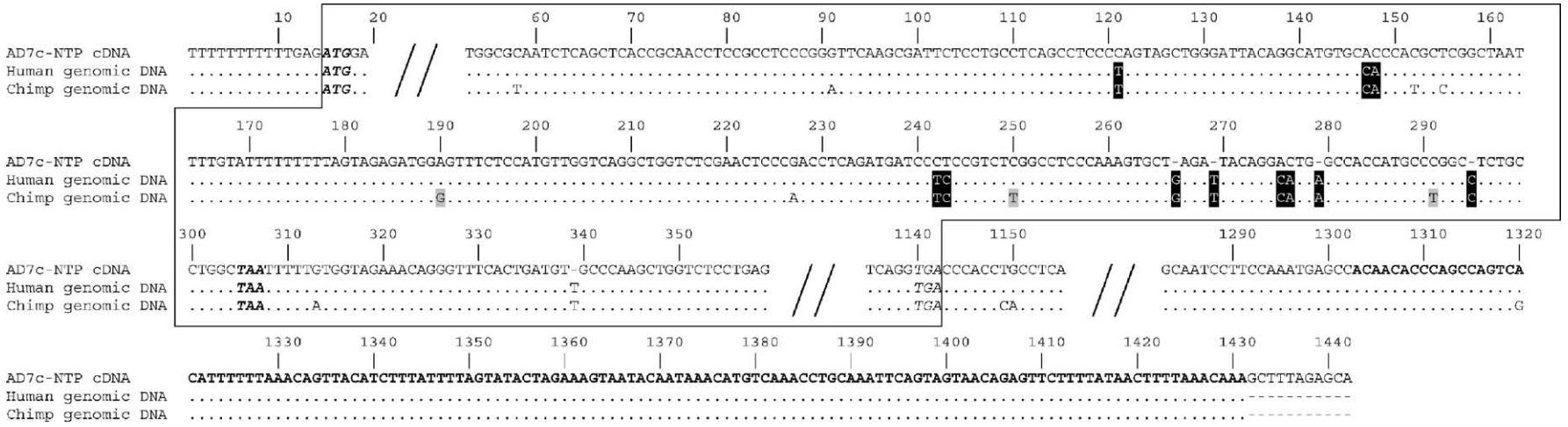

Fig. 1. Sequence comparison of relevant human AD7c-NTP cDNA (GenBank accession number AF010144) and human and chimpanzee orthologous genomic loci (chr1:23777307–23778744 and chr1:24155382–24156812, respectively). The predicted longest "ORF" is framed. The non-repetitive sequence within the cDNA's putative 3′ UTR is shown in bold. Letters on a black background mark 11 nucleotides, that are identical between the human and chimpanzee genomic sequences, but differ from the human AD7c-NTP cDNA. Three letters on a gray background show non-synonymous mutations between the human and chimpanzee genomes, while 5 positions (unmarked) would not alter the amino acids of the hypothetical 98 aa ORF. Most of the cDNA covering the longer "ORF" between positions 360 and 1134 is not shown. This region contains eleven additional mismatches when compared to the human genomic sequence. The 3′-most eleven nucleotides of the cDNA cannot be found in the genomic sequences. Nucleotides identical to the cDNA sequence are symbolized by dots. Hyphens represent gaps. In-frame start and stop codons based on the genomic sequences (ATG. pos. 15–18 and TAA, pos. 305–308) are depicted in bold and italic. The TGA stop codon (pos. 1140–1142) based on the cDNA sequence is shown in italics.

but sufficient part of the presumed 3′ untranslated region of the cDNA reported (AF010144, positions 1303–1431, Fig. 1) is identical to the human genomic sequence and also present in chimpanzee, based on which we extracted the latter orthologous sequence, as these nucleotides are unique and not contributed by repetitive elements. Assuming causality (which can be argued), Alzheimer's disease would be predictable for individuals carrying this allele and there should be no expression of this allele in controls.

There are additional serious problems: The detection of the AD7c-NTP mainly consisting of the Alu-cassette's transcript was performed by Northern Blot analysis with the radio labeled Alu-containing insert as the probe. Since over 1000 human transcripts contain an Alu-cassette (W.M., unpublished observation), shouldn't this give rise to a smear of transcripts that contain Alu elements? For RT-PCR, internal Alu-derived primers were used amplifying a short fragment of neighboring parts of AluSg and AluJo subfamily members. There is an estimated copy number of 115,000 AluSg and 145,000 AluJo elements, respectively, within the human genome and a tendency of Alu elements to colocalize. With many of them present in 5′ or 3′ UTRs or CDSs of mRNAs, wouldn't one expect again an undefined smear instead of a single band on the gel? Unfortunately, for both experiments data were not presented. The fact that an antibody against an ORF contributed by Alu sequences recognizes a protein is not surprising in light of the exonization of Alu elements in primates [2,3]. The antibody against the recombinant AD7c-NTP protein, whichever reading frame it represents, is bound to identify a number of proteins that contain amino acid sequences contributed by Alu elements (e.g., Ref. [6]). In fact, Western blots (Fig. 2D in Ref. [4]) not only identify bands for the expected molecular weight of AD7c-NTP (41 kD) but also additional ones with higher and lower molecular weights, in many instances even more prevalent than the putative 41 kD protein. Such targets might also have been detected in the "7c Gold"/"AlzheimAlert" assay reported [7]. Nevertheless, the short ORF in this region is conserved between human and chimpanzee and presumably also encoded by the cDNA. Even if the cloned cDNA expressed in *E. coli* actually contains the differences (and the differences between the cDNA and genomic sequence are not due to sequencing errors), still a protein is obtained that over the N-terminal 84 amino acid codons is virtually identical to the one predicted from the genomic sequences. If the 10.4 kD hypothetical protein is expressed in the human brain, one would expect cross-reactivity between this protein and the antibody generated from the cDNA in any event.

Two recent publications [8,9] from other laboratories refer to the results presented in [4]. One study identified a novel gene (PDLIM5) and concludes that it is a homolog of AD7c-NTP [8]. PDLIM5 consists of three exons (inspection of the genomic locus discloses the possibility of additional distal exons). Part of an inverted Alu element likely has been exonized as exon 3, a frequent mechanism of including Alu derived sequences into the open reading frame of proteins [2]. This is, as indicated above, the sole reason for "homology" and substantiates the suspicion that antibodies against recombinant AD7c-NTP potentially cross-react with numerous human proteins. The other paper [9] solely reports the chromosomal location of AD7c-NTP and predicts that the deduced protein contains transmembrane domains. The data we are presenting here makes it mandatory to reassess the claims in all publications associated with AD7c-NTP including the ones connecting the protein to the etiology or diagnosis of Alzheimer's disease or a function in the induction of apoptosis [4,6–18].

References

[1] J. Brosius, S.J. Gould, On "genomenclature": a comprehensive (and respectful) taxonomy for pseudogenes and other "junk DNA", Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 10706–10710.

[2] R. Sorek, G. Ast, D. Graur, Alu-containing exons are alternatively spliced, Genome Res. 12 (2002) 1060–1067.

[3] S.S. Singer, D.N. Mannel, T. Hehlgans, J. Brosius, J. Schmitz, From "junk" to gene: curriculum vitae of a primate receptor isoform gene, J. Mol. Biol. 341 (2004) 883–886.

[4] S.M. de la Monte, K. Ghanbari, W.H. Frey, I. Beheshti, P. Averback, S.L. Hauser, H.A. Ghanbari, J.R. Wands, Characterization of the AD7c-NTP cDNA expression in Alzheimer's disease and measurement of a 41-kD Protein in cerebrospinal fluid, J. Clin. Invest. 100 (1997) 3093–3104.

[5] E.S. Lander, et al., Initial sequencing and analysis of the human genome, Nature 409 (2001) 860–921.

[6] J. Hoenicka, M. Arrasate, J.G. de Yebenes, J. Avila, A two-hybrid screening of human Tau protein: interactions with Alu-derived domain, NeuroReport 13 (2002) 343–349.

[7] S.M. de La Monte, J.R. Wands, The AD7c-NTP neuronal thread protein biomarker for detecting Alzheimer's disease, J. Alzheimer's Dis. 3 (2001) 345–353.

[8] M. Wu, Y. Li, C. Ji, J. Xu, H. Zheng, X. Zou, S. Gu, Y. Lou, Y. Xie, Y. Mao, Cloning and identification of a novel human gene PDLIM5, a homolog of AD-associated neuronal thread protein (AD7c-NTP), DNA Seq. 15 (2004) 144–147.

[9] J.H. Gui, J.Y. Liu, L.T. Jia, D.H. Chou, W.Z. Yu, J. He, The chromosomal location of AD7c-NTP gene and the prediction of transmembrane domains of its deduced protein, Xibao Yu Fenzi Mianyixue Zazhi 20 (2004) 604–607.

[10] S.M. de la Monte, R.I. Carlson, N.V. Brown, J.R. Wands, Profiles of neuronal thread protein expression in Alzheimer's disease, J. Neuropathol. Exp. Neurol. 55 (1996) 1038–1050.

[11] S.M. de la Monte, W. Garner, J.R. Wands, Neuronal thread protein gene modulation with cerebral infarction, J. Cereb. Blood Flow Metab. 17 (1997) 623–635.

[12] K. Ghanbari, H.A. Ghanbari, A sandwich enzyme immunoassay for measuring AD7c-NTP as an Alzheimer's disease marker: AD7c test, J. Clin. Lab. Anal. 12 (1998) 223–226.

[13] H. Ghanbari, K. Ghanbari, I. Beheshti, M. Munzar, A. Vasauskas, P. Averback, Biochemical assay for AD7c-NTP in urine as an Alzheimer's disease marker, J. Clin. Lab. Anal. 12 (1998) 285–288.

[14] P.J. Kahle, M. Jakowec, S.J. Teipel, H. Hampel, G.M. Petzinger, D.A. Di Monte, G.D. Silverberg, H.J. Moller, J.A. Yesavage, J.R. Tinklenberg, E.M. Shooter, G.M. Murphy, Combined assessment of

Tau and neuronal thread protein in Alzheimer's disease CSF, Neurology 54 (2000) 1498–1504.
[15] J.J. Higgins, D.R. Rosen, J.M. Loveless, J.C. Clyman, M.J. Grau, A gene for nonsyndromic mental retardation maps to chromosome 3p25-pter, Neurology 55 (2000) 335–340.
[16] S.M. de la Monte, J.R. Wands, Alzheimer-associated neuronal thread protein-induced apoptosis and impaired mitochondrial function in human central nervous system-derived neuronal cells, J. Neuropathol. Exp. Neurol. 60 (2001) 195–207.
[17] S.M. de la Monte, J.R. Wands, Neurodegeneration changes in primary central nervous system neurons transfected with the Alzheimer-associated neuronal thread protein gene, Cell. Mol. Life Sci. 58 (2001) 844–849.
[18] S.M. de la Monte, G.J. Chen, E. Rivera, J.R. Wands, Neuronal thread protein regulation and interaction with microtubule-associated proteins in SH-Sy5y neuronal cells, Cell. Mol. Life Sci. 60 (2003) 2679–2691.