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Hippocampus-specific deficiency in RNA editing of GluA2 in Alzheimer's disease

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ABSTRACT

Adenosine to inosine (A-to-I) RNA editing is a base recoding process within precursor messenger RNA, catalyzed by members of the adenosine deaminase acting on RNA (ADAR) family. A notable example occurs at the Q/R site of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid glutamate receptor subunit GluA2. Abnormally, low editing at this site leads to excessive calcium influx and cell death. We studied hippocampus and caudate samples from Alzheimer's disease (AD) patients and age-matched healthy controls, using direct sequencing and a high accuracy primer-extension technique to assess RNA editing at the Q/R GluA2 site. Both techniques revealed lower, more variable RNA editing in AD, specific to the hippocampus and the GluA2 site. Deficient editing also characterized the hippocampus of apolipoprotein ε4 allele carriers, regardless of clinical diagnosis. In AD, messenger RNA expression of neuronal markers was decreased in the hippocampus, and expression of the Q/R-site editing enzyme ADAR2 was decreased in caudate. These findings provide a link between neurodegenerative processes and deficient RNA editing of the GluA2 Q/R site, and may contribute to both diagnosis and treatment of AD.

1. Introduction

Adenosine to inosine (A-to-I) RNA editing is a dynamic, site-specific base recoding process that occurs within precursor messenger RNA (mRNA), and is catalyzed by members of the adenosine deaminase acting on RNA (ADAR) family (Bass, 2002; Valente and Nishikura, 2005). ADAR-mediated RNA editing is particularly prevalent in the human brain (Chen et al., 2000; Paz et al., 2007; Valente and Nishikura, 2005), and occurs at both protein-coding and noncoding regions of the RNA molecule.

A notable example of A-to-I RNA editing at a coding region is the Q/R site of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor subunit GluA2, which undergoes extensive (>99.9%) ADAR2- mediated editing at residue 607, where the genomic glutamine (Q607) is converted to an arginine (R) codon (Geiger et al., 1995; Jonas and Burnashev, 1995). Low A-to-I editing levels at this site were observed in spinal motor neurons of individuals with sporadic amyotrophic lateral sclerosis (ALS), providing a putative mechanism for neuronal death in this fatal paralytic disease (Kawahara et al., 2004).

In vitro and in vivo studies in rodents indicate that editing at the Q/R site of GluA2 controls cellular permeability to calcium (Ca^{2+}); reduced editing at this site results in excessive Ca^{2+} influx and neuronal demise (Brusa et al., 1995; Feldmeyer et al., 1999; Higuchi et al., 2000; Peng et al., 2006). Dysregulated GluA2 editing may therefore characterize neuronal populations subject to significant cellular demise.

Alzheimer's disease (AD) is characterized clinically by a progressive decline in cognitive abilities and pathologically by

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Table 1
Clinicopathological summary of subjects included in the study

Tissue	Group	N	Mean age ± SD	Gender (%)	ApoE allele frequencies (%)		Braak score frequencies (N)			
				F/M	ε3	ε4	0–2	4	5	6
HIPP	HC	15	80.4 ± 12.5	90	95.8	4.2	15	0	0	0
	AD	22	79.7 ± 10.8	120	57.5	42.5	0	3	16	3
CAU	HC	17	80 ± 11.8	89	95.8	4.2	17	0	0	0
	AD	22	79.7 ± 10.8	120	57.5	42.5	0	3	16	3

Key: AD, Alzheimer's disease; ApoE, apolipoprotein; CAU, caudate; F, female; HC, healthy controls; HIPP, hippocampus; M, male; N, number of individuals; SD, standard deviation.

substantial neuronal and synaptic loss, diminished neuronal metabolism, senile plaques, neurofibrillary tangles, and aberrant function of multiple neurotransmitter systems (Parihar and Hemnani, 2004). Postmortem and imaging studies indicate that the hippocampus plays a central role in AD pathophysiology. Changes in hippocampal volume, shape, and function precede disease onset, and differentiate the course of the disease from normal aging (Wang et al., 2003). While the etiology of AD is unclear, the ε4 allele of the apolipoprotein (ApoE) gene has emerged as a prominent genetic risk factor. Individuals with 1 or 2 copies of ε4 allele have a higher risk of developing AD, compared with carriers of the ε2 or ε3 isoforms, and develop symptoms at an earlier age (Cedazo-Minguez, 2007).

Despite the putative relevance of GluA2 Q/R site RNA editing to neurodegenerative processes, few studies specifically examined RNA editing in AD patients. A pioneering study by Akbarian et al. (1995) pointed to a decrease in GluA2 Q/R editing in the prefrontal cortex of individuals with AD but did not examine RNA editing or ADAR expression changes in the hippocampus. In the present study, we assessed GluA2 Q/R site RNA editing levels postmortem in the hippocampus of AD patients as well as in the striatum, a brain region relatively spared by AD. Furthermore, we asked whether ApoE genotype and RNA editing at this site interact to produce a disease phenotype. Finally, we asked whether the mRNA expression of RNA editing enzymes was altered in the hippocampus and striatum of patients with AD.

2. Methods

2.1. Subjects

Human brain tissue was obtained through the rapid autopsy program of the Netherlands Brain Bank (Coordinator: Dr I. Huitinga). The Netherlands Brain Bank abides by all local ethical legislation. All tissues were obtained with informed consent of the donor or next of kin to perform brain autopsy and the subsequent use of brain tissue for scientific purposes. Permission to use the medical records was also requested in advance.

Hippocampal and striatal samples from 22 AD patients (12 females) and 17 nondemented healthy controls (8 females) were studied. Careful selection and matching were performed based on medical and neuropathological records. Subjects in the AD group had an antemortem diagnosis of probable AD confirmed by postmortem pathology (Braak stage 4–6, Braak and Braak, 1995). Nondemented controls had no neurologic or psychiatric diagnosis antemortem, also confirmed by pathologic examination (Braak stages 0–2). Postmortem delay was on average 08:40 hours ± 05:43 standard deviation for the control subjects and 04:45 hours ± 01:34 standard deviation for AD subjects (see Table 1 for further details).

2.2. Sample preparation

RNA was extracted using RNEasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. Samples were treated with RNase-free DNase (Qiagen). The reverse transcription reaction was performed using 1 λ of total RNA and the High Capacity cDNA RT Kit w RNAse I (Applied Biosystems, Foster City, CA, USA). Double-distilled H_2O (DDW) was added to reach a final volume of 40 λ per sample. Complementary DNA (cDNA) obtained had a final concentration of 25 ng/λ.

2.3. Determining RNA editing levels

2.3.1. Direct sequencing

Sanger direct sequencing was performed as previously described (Barbon et al., 2003). Briefly, following amplification of the region containing the editing site, a pool of GluA2 cDNA was obtained in which both the edited and unedited mRNA forms were co-expressed. The polymerase chain reaction (PCR) products were sequenced using a genetic analyzer 3100 (Applied Biosystems) and read using Sequencher 4.2 software (Gene Codes Corp, Ann Arbor, MI, USA). The edited nucleotide appeared as overlapping A/G peaks: A from unedited transcripts and G from the edited ones. The percentage of edited mRNA molecules in a pool of specific GluR mRNAs can be determined by calculating the peak area of the edited nucleotide (G) versus the sum of G and A peak areas using Discovery Studio gene 1.5 software (Accelrys Inc, San Diego, CA, USA). Previous studies confirmed that the DS gene quantification method is reliable as compared with determination of editing from cloned sequences (Barbon et al., 2003; Paz et al., 2007).

2.3.2. Sequenom mass array

The Sequenom assay was performed as previously described (Jurinke et al., 2005; Koren-Michowitz et al., 2008). Briefly, PCR amplifications were carried out in standard 384-well plates, in a 5 μL final volume containing 20 ng of template cDNA, 0.1 U of Taq polymerase (HotStarTaq; Qiagen), 0.2 mM of each dNTP, 200 nmol of each primer, 1 mM MgCl2, and 1× HotStar buffer. To remove the nonincorporated dNTPs, PCR products were incubated with Shrimp alkaline phosphatase (0.3 U, in a total volume of 7 μL; 20 minutes at 37 °C and 5 minutes at 85 °C). The MassEXTEND assay was then conducted in a 9 μL final volume containing 1 μM extension primer, 0.2 μL of termination mix (50 nM each of ddA, dG, ddT, and ddC), and 1.25 U ThermoSequenase (Sequenom, San Diego, CA, USA) in 0.22× PCR buffer. Following this step, 3 μg MassEXTEND cleanup resin (Sequenom) and 16 μL DDW were added to remove extraneous salts. Samsung nanodispenser was used to apply 15 nL of the extension products from each well of the sample plate onto the SpectroChips (Sequenom). Mass spectra were recorded on a Bruker Biflex MALDI-TOF mass spectrometer (Bremen, Germany) operated in the linear mode, and were finally analyzed by MassARRAY Typer software (Sequenom). Analyzer accuracy rate, as reported by the manufacturer, varies between 97% and 99%. Editing results were presented by mass spectrometer peaks and calculated as the area under the spike. Capture and extension primers for the GluA2,