Short communication

Age-related gene-specific changes of A-to-I mRNA editing in the human brain

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ARTICLE INFO

ABSTRACT

A-to-I editing is an adenosine-to-inosine modification of mRNA particularly widespread in the human brain, where it affects thousands of genes. A growing body of evidence suggests that A-to-I RNA editing is necessary for normal development and maintenance in mammals and that its deficiencies contribute to a number of pathological states. In this study, we examined whether mRNA editing levels of two mRNA species, CYFIP2 and GABRA3, change with aging. CYFIP2 has been implicated in synaptic maintenance, while GABRA3 is a GABA receptor subunit, a part of the major inhibitory neurotransmitter system in the CNS. The levels of mRNA editing were assessed in cortex samples of 20 subjects 22–102 years old. The data show an age-dependent statistically significant decrease in editing in CYFIP2. GABRA3 editing remained much more stable with age, implying that age-related decline of RNA editing is gene-specific. This is the first report of age-dependent decline in A-to-I editing. Further examination of these and other vulnerable genes may reveal specific RNA editing mechanisms that contribute to the aging phenotype.

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Adenosine-to-inosine (A-to-I) RNA editing is a post-transcriptional processing pathway particularly widespread in the human brain wherein adenosine in pre-mRNA is modified to yield inosine, which is equivalent of guanosine for the splicing and translational machineries. This process is catalyzed by the members of the double-stranded RNA (dsRNA)-specific ADAR (adenosine deaminase acting on RNA) family (Bass, 2002). In the past few years, bioinformatic and experimental studies have revealed tens of thousands of editing sites affecting over 1600 different genes (Athanasiadis et al., 2004; Blow et al., 2004; Kim et al., 2004; Levanon et al., 2004; Morse and Bass, 1999). Deregulation (or dysregulation) of RNA editing has been linked to a few diseases of the central nervous system (Maas et al., 2006), such as depression (Gurevich et al., 2002; Niswender et al., 2001), epilepsy (Brusa et al., 1995), glioblastoma (Cenci et al., 2008; Maas et al., 2001; Paz et al., 2007) and amyotrophic lateral sclerosis (Kawahara et al., 2007). It is thus conceivable that if RNA editing becomes deregulated with age, then it may in part explain the decline in some brain functions attributed to aging.

Changes in other types of mRNA editing have been previously discovered. These include developmental and age-related changes for a much more rare type of RNA editing such as (C-to-U) of the apoB in mice which are believed to represent a regulated process of lipoprotein biogenesis (Higuchi et al., 1992) and other possible editing type (van Leeuwen et al., 1998). However, although A-to-I editing is much more abundant, there is almost no knowledge about its regulation during aging.

In the present study, we tested whether levels of RNA editing change with age by examining two carefully selected transcripts, GABRA3 and CYFIP2. CYFIP2 is a p53-inducible protein (Jackson et al., 2007), which has been implicated in synaptic maintenance (Schenck et al., 2001), and its editing levels are regulated in murine development (Wahlstedt et al., 2009). GABRA3 is a variant of the alpha subunit of a GABA-A receptor, a part of the major inhibitory neurotransmitter system in the CNS (Akabas and Ronald, 2004). GABRA3 RNA editing is also tightly regulated (increased A-to-I) during early development, and the edited and the non-edited form may have different functional profiles in the mouse (Rula et al., 2008), (Wahlstedt et al., 2009). CYFIP2 and GABRA3 transcripts were selected because editing in both is regulated during development, both function in the brain, and both are located in extremely conserved genomic regions suggesting an important function. In addition, both transcripts have shown high levels of RNA editing in young adult individuals and each contained a single edited site thus allowing for straightforward quantitative analysis.

Levels of RNA editing were measured in 25 frontal cortex samples from individuals aged 22–102 years old (Table 1) by quantitative analysis of the sequencing chromatograms of the RT-PCR products (see Materials and Methods on-line for detailed description of the samples and the procedures). The level of editing

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Table 1
List of samples.

Age	Cause of death	Gender	Editing level	
			GABRA	CYFIP2
22	Suicide	m	0.9	0.85
32	Suicide	f	0.9	0.88
33	Suicide	m	0.92	0.85
34	Cystic fibrosis	m	0.95	0.65
40	Suicide	m	0.94	0.85
44	**Skin cancer/ventilator**	**f**	**0.6**	**0.35**
47	Heart failure	?	0.95	0.85
47	**Hypoxia ischemia**	**m**	**0.6**	**0.8**
51	Heart failure	m	0.94	0.8
56	Bone cancer	m	0.95	0.6
57	Heart failure	f	0.9	0.63
60	Heart failure	f	0.9	0.77
67	Heart failure	m	0.9	0.7
76	Heart failure	m	0.88	0.75
76	Unknown	m	0.95	0.6
77	Heart failure	m	0.9	0.73
77	Heart failure	m	0.9	0.75
80	Heart failure	?	0.91	0.725
80	Heart failure	f	0.95	0.7
88	Heart failure	f	0.9	0.78
88	Heart failure	f	0.9	0.6
89	Heart failure	?	0.84	0.6
89	Leukemia	f	0.9	0.55
95	Heart failure	?	0.9	0.67
97	Heart failure	f	0.9	0.725
97	Heart failure	f	0.93	0.56
102	Heart failure	m	0.85	0.675

Samples considered outliers are shown in bold.

as a function of age is graphically presented in Fig. 1. The decrease of CYPFIP2 with age is statistically significant using both Pearson's ($p = 0.048$) and Spearman's ($p = 0.006$) tests, while this is not true for GABRA ($p = 0.28$, Spearman test; Pearson not applicable since the data was not normally distributed).

Visual inspection of Fig. 1 reveals that two samples are clear outliers (marked yellow, these same samples are shown in
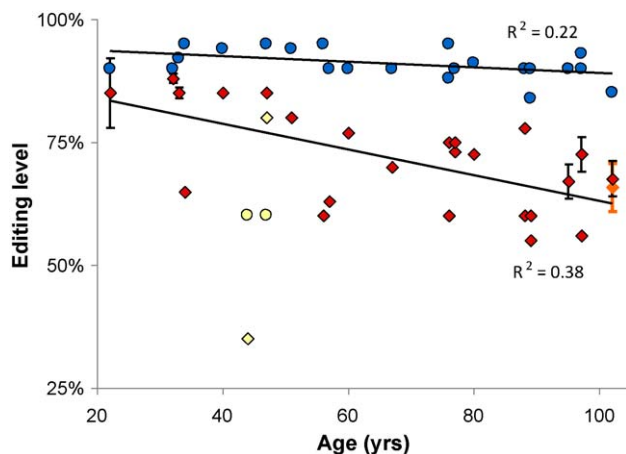

Fig. 1. Levels of editing of the CYFIP2 (diamonds) and GABRA3 (circles) mRNAs as a function of age. While the decrease of CYFPI level with age is statistically significant, there is no significant decrease in the editing levels of GABRA. GABRA decrease becomes significant upon removal of two outlier samples (yellow symbols). These two samples were considered outliers as they lie further than 3 standard deviations away from average. Regression lines constructed in Excel for data with outliers excluded. Error bars show formal standard deviations for samples where two or more measurements were taken. The orange data point represents an average of three different areas, each with duplicate measurements, of the 102 yo individual (this data point was not included in regression analysis). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

boldface in Table 1). In both samples, at least one value (CYPFIP2 or GABRA3 editing level) is more than 3 standard deviations away from the regression line (constructed for all samples). Interestingly, both outlier samples originate from patients who likely suffered unusually prolonged or disruptive treatment/pathology affecting the brain: one patient spent a full month on a ventilator until her death, and in the other one brain tissue was subject to ischemia. These observations imply that aging *per se* is probably not the only factor affecting RNA editing. We therefore decided to re-evaluate the data upon removal of the outliers. As expected, removal of outliers improved the significance of the CYFIP2 trend according to both tests, Pearson's ($p = 0.001$) and Spearman's ($p = 0.002$). Furthermore, now we were able to detect a statistically significant decrease in GABRA3 editing ($p = 0.035$ and 0.043, correspondingly), though the GABRA3 slope is four times as moderate as that of CYPFIP2, implying that age-related changes in the levels of editing are gene-specific.

We further inquired whether the trends we see could have been confounded by the fact that most of our younger samples originate from suicide victims. Perhaps suicidal behavior, not their young age could have been related to the levels of editing in their brain. Analysis shows, however, that the trend for CYPFIP2 remains statistically significant even if suicide victims are removed from the dataset, ($p = 0.007$ using Pearson's and 0.016 using Spearman's test), though the correlation between GABRA and age loses significance.

Previous work by others showed constant levels of A-to-I editing with age. In a mouse model, aging failed to result in a change in AMPA glutamate receptors editing (Carlson et al., 2000). In contrast, our data imply that A-to I editing declines with aging in a gene-specific manner (CYFIP2 affected much more profoundly than GABRA3). Of note, editing of CYFIP2 is performed by the enzyme ADAR2 (Nishimoto et al., 2008; Riedmann et al., 2008), while GARBA3 is a substrate for editing by both ADAR1 and ADAR2 (Ohlson et al., 2007), thus the differences we observe might reflect different changes in the activities or abundance of these enzymes. The fact that levels of RNA editing level of GABRA3 is much lower than that of CYFIP2, suggests that an overall non-specific age-related decline alone cannot account for the changes.

This work is merely the first step in exploring still one more dimension of the human aging process, i.e. changes in the RNA editing levels. Recent technological progress in the RNA editing field opens exciting opportunities of exhaustive, whole-genome studies of RNA editing changes (Li et al., 2009). This approach can hopefully be used to monitor editing levels in all editing sites across the whole human genome simultaneously in multiple samples. The results presented in our report make a case for using these novel approaches to study aged-related changes in RNA editing.

Note: As this manuscript was under review, a newly published linkage analysis study (Sebastiani et al., 2009) reported strong evidence for association of polymorphisms in ADARB1 and ADARB2 with extreme old age. This finding implies that the age-related changes that were observed may potentially be a part of whole-genome changes of RNA editing, which do not merely accompany, but actually cause certain processes that limit human longevity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mad.2010.06.001.

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