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CRTC1 gene is differentially methylated in the human hippocampus in Alzheimer's disease

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Abstract

Background: *CRTC1* (*CREB* regulated transcription coactivator 1) gene plays a role in synaptic plasticity, learning and long-term memory formation in the hippocampus. Recently, *CRTC1* has been shown to be downregulated in Alzheimer's disease (AD). Nevertheless, the mechanisms underlying *CRTC1* dysregulation in AD remain unclear.

Methods: To understand better the epigenetic mechanisms regulating *CRTC1* expression that may be altered in AD, we profiled DNA methylation at CpG site resolution by bisulfite cloning sequencing in two promoter regions (referred to as Prom1 and Prom2) of the *CRTC1* gene in human hippocampus from controls and AD cases. Next, we correlated DNA methylation levels with AD-related pathology, i.e., β -amyloid and phosphorylated-tau (p-tau) burden and also measured *CRTC1* mRNA levels by RT-qPCR.

Results: Methylation levels were lower in AD cases as compared to controls within both promoter regions (Prom1: 0.95 % vs. 5 %, p -value < 0.01 and Prom2: 2.80 % vs. 17.80 %, p -value < 0.001). Interestingly, *CRTC1* methylation levels inversely correlated with AD-related neuropathological changes, particularly with p-tau deposition ($r_{\text{Spearman}} = -0.903$, $p < 0.001$). Moreover, a 1.54-fold decrease in *CRTC1* mRNA levels was observed in hippocampus of AD cases compared to controls ($p < 0.05$) supporting the notion that *CRTC1* is downregulated in the AD hippocampus.

Conclusions: DNA methylation levels within two distinct promoter regions of the *CRTC1* gene were decreased in human hippocampus affected by AD compared with controls and methylation within Prom1 showed a strong inverse correlation with p-tau deposition. Further studies are guaranteed to elucidate the precise role that *CRTC1* methylation plays in AD pathophysiology.

Keywords: *CRTC1*, Epigenetics, DNA methylation, Alzheimer's disease, Hippocampus

Background

CRTC1 (*CREB* regulated transcription coactivator 1) [EMBL: BC017075] plays a critical role as coactivator of the *CREB* (cAMP-responsive element binding)-dependent gene transcription pathway [1], which in turn is essential to synaptic plasticity, learning and long-term memory formation in the hippocampus [2–7]. *CRTC1* is highly expressed in brain, particularly in hippocampal neurons [8, 9], where it translocates from synapses and dendrites

to the nucleus upon the concomitant activation of calcium and cAMP signaling pathways [6, 10, 11]. Moreover, it has been shown that *CRTC1* is involved in dendritic growth of developing cortical neurons [12]. The relevance of this gene to brain function is additionally supported by experiments performed on knockout mouse models. Mice lacking *Crtc1* develop psychomotor retardation along with neurobehavioral endophenotypes. Interestingly, these mice show reduced dopamine and serotonin turnover in the prefrontal cortex and decreased expression of several downstream genes involved in neuroplasticity [13]. In addition, *CRTC1* was recently identified as a modulator of mitochondrial metabolism and longevity [14, 15].

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All this evidence makes *CRTCI* an intriguing candidate to be examined in neurodegenerative disorders at the molecular level [16]. Indeed, the role of *CRCTI* in Alzheimer's disease (AD) has been previously studied in mouse models. Gene transcription mediated by *CRTCI* is impaired in neurons and brain from APP (Sw, Ind) transgenic mice, an AD mouse model overexpressing the human β -amyloid precursor protein (APP) [PDB: E9PG40] carrying human mutations [17, 18]. Remarkably, β -amyloid-induced spatial learning and memory deficits in APP (Sw, Ind) transgenic mice are reversed by restoring a specific subset of *CRTCI* target genes [19]. However, studies performed on postmortem human brain samples are still very scarce. In this regard, a recent report shows that *CRTCI*-dependent genes and *CRTCI* protein [PDB: Q6UUV9] levels are significantly reduced in human hippocampus at intermediate and advanced (IV-VI) Braak pathological stages [19]. Nevertheless, the specific mechanisms underlying *CRTCI*-dependent gene transcriptional disruption in AD still remain unclear.

DNA methylation is a widely studied epigenetic mechanism that contributes to regulate gene expression. The addition of a methyl group to the 5-carbon position of the cytosine-phosphate-guanine (CpG) sites, mostly located at regulatory elements in the genome, modulates the transcription of nearby or related genes. Interestingly, DNA methylation patterns change during the ageing process [20–22] and may have an impact on gene expression in the human brain [23]. In addition, aberrant DNA methylation patterns are involved in an increasing number of common diseases [24, 25], including neurodegenerative conditions. Based on that evidence, we hypothesized that certain regulatory elements of the *CRTCI* gene may be differentially methylated in the hippocampus of AD patients. Hence, we aimed to profile DNA methylation levels by bisulfite cloning sequencing in two distinct promoter regions of *CRTCI* in hippocampal samples from a cohort of neuropathologically defined “pure” AD cases and controls. We also explored the relationship between DNA methylation levels and AD-related neuropathological changes. Next, *CRTCI* mRNA expression was assessed in AD and control hippocampus.

Methods

Human brain samples

We conducted an observational, case-control study comparing postmortem hippocampal samples from AD patients and control hippocampus. Frozen postmortem hippocampal samples from 30 Alzheimer's disease (AD) cases and 12 controls were provided by the Navarrabiomed Brain Bank. After death, half brain specimens from donors were cryopreserved at -80°C .

Immunostaining protocol

Formalin-fixed (4 %), paraffin-embedded tissue from mid-hippocampus region was cut (5-mm sections) and placed on StarFrost Microscope Slides. After deparaffinizing, endogenous peroxidase activity was quenched by incubation with 0.3 % (vol/vol) H_2O_2 in methanol for 30 minutes at room temperature. Antigen retrieval was performed by soaking the sections in 10-mM citrate buffer pH 6.0, heated and boiled for 10 minutes in a microwave oven.

Assessment of β -amyloid deposition was carried out with a mouse monoclonal (S6F/3D) anti β -amyloid antibody (Leica Biosystems Newcastle Ltd, Newcastle upon Tyne, UK) which identified the presence of amyloid pathology.

Evaluation of neurofibrillary pathology was performed with a mouse monoclonal antibody anti-human PHF-TAU, clone AT-8, (Tau AT8) (Innogenetics, Ghent, Belgium), which identified hyperphosphorylated tau (p-tau) [26] in the shape of dystrophic neurites and neurofibrillary tangles. The reaction product was visualized using an automated slide immunostainer (Leica Bond Max) with Bond Polymer Refine Detection (Leica Biosystems, Newcastle Ltd). Omission of the primary antibody was used as a negative control for all stainings.

Neuropathological assessment

Neuropathological examination was completed following the usual recommendations [27] and assessment of AD was performed according to the updated National Institute on Aging-Alzheimer's Association guidelines [26]. Samples were further classified, based on the ABC score, into four groups: control ($n = 12$), initial AD (including A1B1C1, A1B2C1, A1B2C2, and A1B2C3 scores) ($n = 9$), intermediate AD (including A2B2C2, A2B2C3, and A2B3C3 scores) ($n = 7$) and advanced AD (including A3B2C1, A3B2C3, A3B3C2, and A3B3C3 scores) ($n = 14$) (Additional file 1: Figure S1). Importantly, to avoid spurious associations, those individuals showing coexisting protein deposits different from p-tau or β -amyloid were not eligible for the study. This approach maximizes chances of finding true associations with AD, even though reducing the final sample size. Neuropathological and demographic features of subjects, including age, gender, ABC score and postmortem interval are listed in Additional file 2: Table S1.

Quantitative assessment of β -amyloid and p-tau deposits in brain tissues

In order to quantitatively assess the β -amyloid and p-tau burden for further statistical analysis we applied a method to quantify protein deposits. This method generates a numeric measurement that reflects the extent of

β -amyloid and p-tau deposition. Sections of the mid hippocampus were examined after performing immunostaining with anti β -amyloid and anti p-tau antibodies as described above. Three pictures were obtained for each immunostained section using an Olympus BX51 microscope at 10x magnification power. Morphological deposits of β -amyloid, as described by Braak and Braak (neuritic, immature and compact plaque), were manually determined and those areas were further edited and analyzed with ImageJ software [28] (Additional file 1: Figure S2). Next, β -amyloid plaque count referred to as amyloid plaque score (APS) and total area of β -amyloid deposition were automatically measured by ImageJ and averaged for each section. Regarding p-tau deposit, pictures were also analyzed with ImageJ software, by adjusting in the threshold color hue (maximum range: 45–197), saturation (maximum range: 7–243) and brightness (maximum range: 50–195), in order to obtain an averaged quantitative measure of the global p-tau deposit for each section (Additional file 1: Figure S2).

***CRTC1* methylation profiling by bisulfite cloning sequencing**

Genomic DNA was isolated from hippocampal tissue by standard methods [29]. Next, 500 ng of genomic DNA was bisulfite converted using the EpiTect Bisulfite Kit (QIAGEN, Redwood City, CA, USA) according to the manufacturer's instructions. Two promoter regions (Prom1 and Prom2) within the *CRTC1* gene were amplified by PCR (Additional file 1: Figure S3). Genomic coordinates were obtained from GRCh37/Hg19 assembly. Primer pair sequences were designed by MethPrimer [30] and are listed in Additional file 2: Table S2. PCR products were cloned using the TopoTA Cloning System (Invitrogen, Carlsbad, CA, USA) and a minimum of 12 independent clones were sequenced for each examined subject and region. Methylation graphs were obtained with the QUMA software [31].

***CRTC1* mRNA expression analysis**

Total RNA was isolated from the 42 hippocampus homogenates using RNeasy Lipid Tissue Mini kit (QIAGEN, Redwood City, CA, USA), following the manufacturer's instructions. Genomic DNA was removed with recombinant DNase (TURBO DNA-free™ Kit, Ambion, Inc., Austin, TX, USA). RNA integrity was checked by 1.25 % agarose gel electrophoresis under denaturing conditions. Concentration and purity of RNA were both evaluated with a NanoDrop spectrophotometer. Only RNA samples showing a minimum quality index (260 nm/280 nm absorbance ratios between 1.8 and 2.2 and 260 nm/230 nm absorbance ratios higher than 1.8) were included in the study. Complementary DNA (cDNA) was reverse transcribed from 1500 ng

total RNA with SuperScript® III First-Strand Synthesis Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) after priming with oligo-d (T) and random primers. RT-qPCR reactions were performed in triplicate with Power SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA, USA) in an Applied 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Sequences of primer pair were designed using the Real Time PCR tool (IDT, Coralville, IA, USA) and are listed in Additional file 2: Table S2. Relative expression level of *CRTC1* mRNA in a particular sample was calculated as previously described [32] and the geometric mean of *GAPDH* and *ACTB* genes were used to normalize expression values.

Statistical data analysis

Statistical analysis was performed with SPSS 21.0 (IBM, Inc., USA). Data represent the mean \pm SEM or median (range), depending on the type of variable. Differences with p -value < 0.05 were considered significant. Statistical significance for expression and bisulfite intergroup differences were assessed by the Mann-Whitney U test and the Kruskal–Wallis test. Spearman's rank correlation coefficient was used to determine correlation between AD-related pathology and methylation levels. When multiple comparisons were performed, as was the case of correlation between methylation levels and AD-related pathological burden, a Bonferroni correction was applied and the significance threshold was set at p -corrected value = 0.008. GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, USA) was used to draw the graphs except for methylation figures that were obtained by QUMA software.

Ethics, consent and permissions

Our study was carried out in accordance with the Declaration of Helsinki and handling of human brain samples was performed according to the current Spanish national legislation (Law 14/2007 and Royal Decree RD1716/2011). The Ethics Committee of the "Complejo Hospitalario de Navarra" approved the use of human subjects for this study (90/2014). Written informed consent was obtained from all subjects or next of kin, previous to brain donation, to perform research projects related to neurodegenerative conditions. The consent form is held by the authors' institution and is available for review by the Editor-in-Chief.

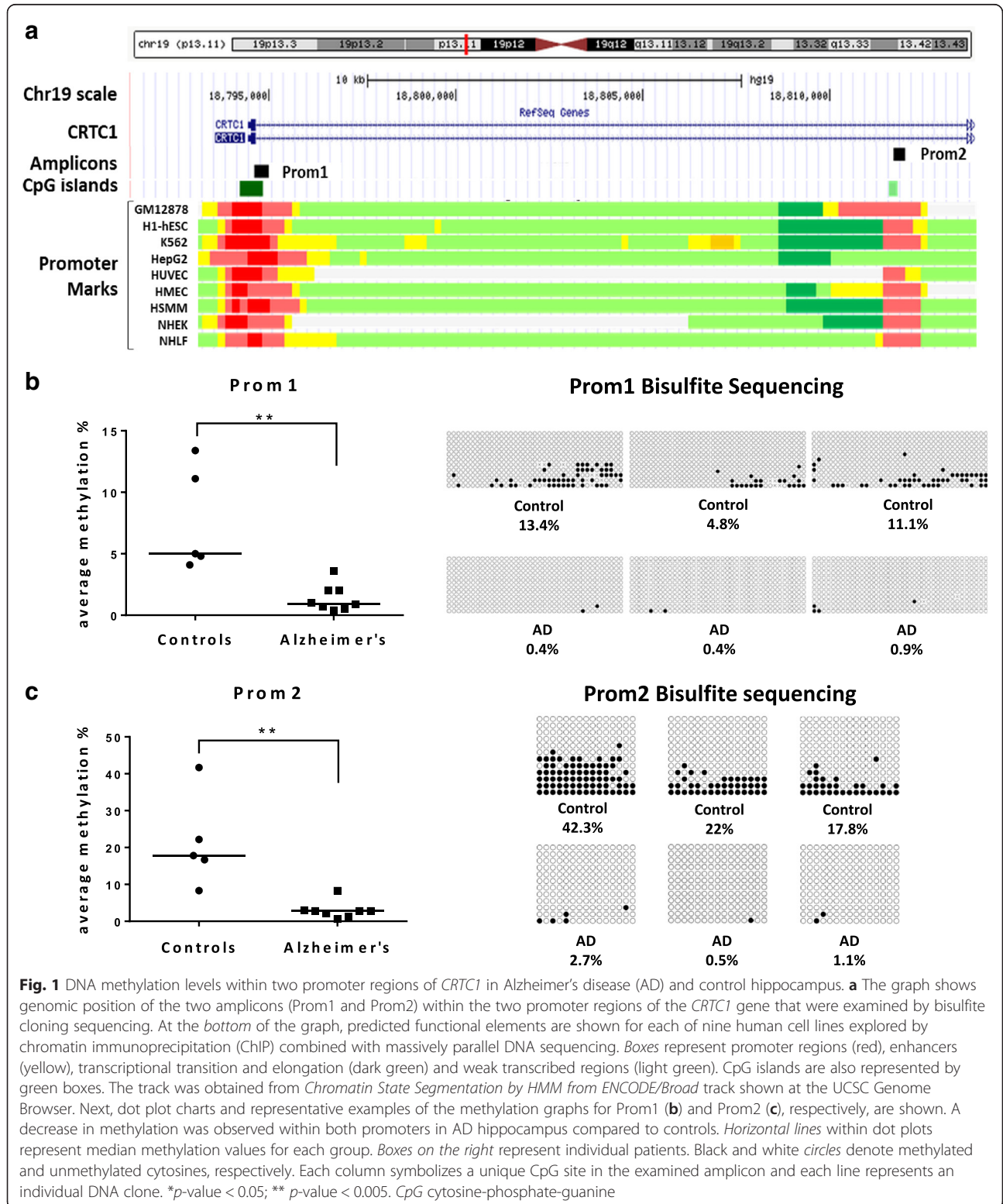
Results

***CRTC1* methylation is decreased in hippocampus of AD cases compared to controls**

To begin to ask whether DNA methylation within *CRTC1* is altered in AD hippocampus, two different promoters of the *CRTC1* gene were examined by bisulfite cloning

sequencing. Promoters were identified by using chromatin immunoprecipitation sequencing (ChIP-seq) data from the track *Chromatin State Segmentation by HMM (Hidden Markov Model) from ENCODE project and Broad*

Institute, shown at the UCSC Genome Browser [33]. This track displays chromatin state segmentation data for each of nine human cell types (Fig. 1a, Additional file 1: Figure S3). We designed primers to amplify two



independent amplicons. Promoter 1 (Prom1) amplicon was placed at the 5' end of *CRTC1*, partially overlapping exon 1 and the beginning of intron 1, and contained 37 CpG sites within the southern shore of a 628 bp-CpG island (*chr19:18794192–18794819*). Whereas promoter 2 (Prom2) amplicon was located within intron 1 and encompassed 15 CpG sites at the southern shore of a 230 bp-CpG island (*chr19:18811562–18811791*) (Fig. 1a, Additional file 1: Figure S3). To sum up, a total of 52 individual CpG sites, representing 63.8 % and 88.2 % of each CpG island, respectively, were analyzed in a subset of five control and eight AD cases. To calculate the average methylation level, DNA methylation percentage was measured at CpG site resolution and further averaged across all the CpG sites for each promoter region and subject. Interestingly, we found that average methylation level at Prom1 was decreased in AD cases compared to controls (0.95 % vs. 5 %, p -value < 0.01) (Fig. 1b). Similarly, average methylation level was significantly decreased within Prom2 in AD cases compared to controls (2.80 % vs. 17.80 %, p -value < 0.001) (Fig. 1c).

***CRTC1* methylation correlates with p-tau burden**

Next, we aimed to correlate DNA methylation levels with AD-related neuropathological changes recorded from the hippocampus sections. In brief, p-tau and β -amyloid burden were measured and averaged by using the ImageJ software (Additional file 1: Figure S2). The amyloid plaque score (APS) was also recorded (Additional file 2: Table S1). Notably, the average methylation level within Prom 1 showed a strong inverse correlation with p-tau deposition ($r_{\text{Spearman}} = -0.903$, $p < 0.001$). Although we found a correlation between Prom1 average methylation level and β -amyloid burden (Table 1), the p -value for this correlation did not reach the Bonferroni-corrected significance threshold (corrected p -value = 0.008). Likewise, average methylation level within Prom2 was no longer correlated with p-tau deposition and β -amyloid burden after applying the Bonferroni correction (Table 1).

***CRTC1* mRNA levels are downregulated in Alzheimer's disease hippocampus**

To test if *CRTC1* was differentially expressed in our cohort, we measured *CRTC1* mRNA expression levels by

RT-qPCR in hippocampal samples from Alzheimer's disease (AD) cases and controls. Four samples did not pass the RNA quality threshold and so were not included in the experiments (see *CRTC1* mRNA expression analysis in the Methods section). Eventually, 26 AD cases were compared to 12 controls. RT-qPCR reactions were performed in triplicate for each sample and repeated twice within independent cDNA sets. Moreover, two different sets of primers (*CRTC1*-qPCR1 and *CRTC1*-qPCR2) were used to increase the reliability of the experiment (Additional file 2: Table S2). A 1.54-fold decrease in *CRTC1* mRNA levels was observed in the hippocampus of AD cases compared to controls ($p < 0.05$) (Fig. 2a). Next, a disease-staging analysis was conducted to investigate changes of *CRTC1* mRNA levels across AD stages. We found that *CRTC1* mRNA levels significantly decreased across AD stages ($p < 0.05$) and post-hoc analysis showed that *CRTC1* expression was significantly reduced at advanced stages compared to controls ($p < 0.05$) (Fig. 2b).

We further tested if differences in *CRTC1* mRNA expression resulted from a decrease in a particular type of *CRTC1* transcript variant. Two different *CRTC1* transcripts were identified in the Reference Sequence (RefSeq) database, i.e., *CRTC1* transcript variant 1 (NM_015321) and transcript variant 3 (NM_001098482), sharing all the exons but for an additional exon (exon 3) belonging to *CRTC1* variant 3 (Fig. 2c). Due to the extra exon 3, we were able to design a primer pair to selectively amplify *CRTC1* variant 3 (Additional file 2: Table S2), whereas a primer pair to amplify only *CRTC1* variant 1 was not able to be designed since all the exons of this variant are shared with *CRTC1* variant 3. We found that levels of *CRTC1* variant 3 mRNA were not different between AD cases and controls ($p = 0.206$), suggesting that significant differences in global *CRTC1* mRNA levels between AD cases and controls might result from a reduction in *CRTC1* variant 1 mRNA.

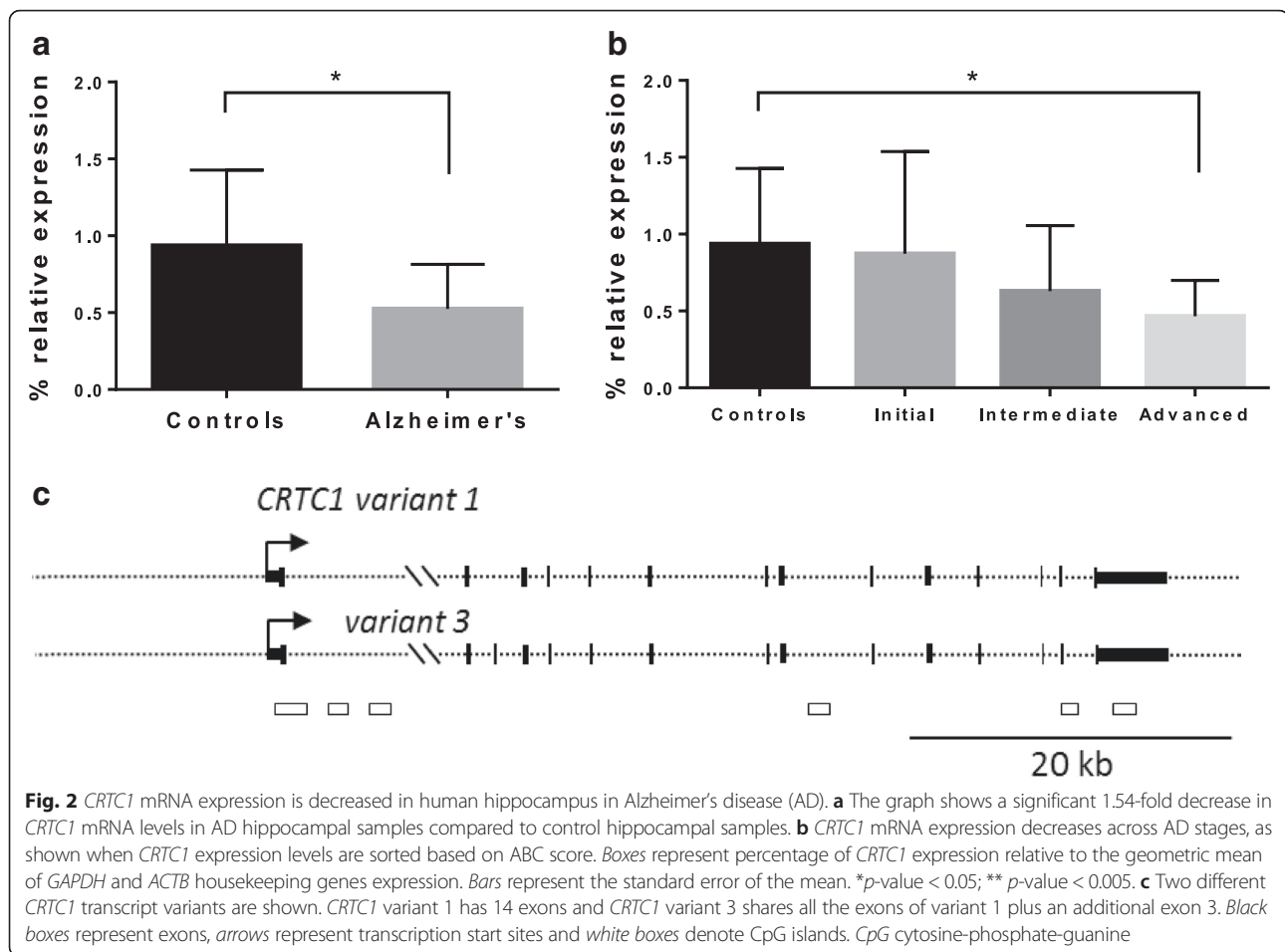
Finally, we studied the relationship between promoter methylation and mRNA expression for the *CRTC1* gene. We tested the correlation between DNA methylation levels and mRNA expression levels. In order to avoid potential bias from disease status, we tested the potential correlation separately in two groups, controls and AD patients. Interestingly, *CRTC1* mRNA expression showed a trend to

Table 1 Correlation tests between *CRTC1* methylation levels and Alzheimer's disease (AD)-related pathological changes

AD-related changes	Prom1 methylation level		Prom2 methylation	
	Spearman coefficient	raw p -value	Spearman coefficient	raw p -value
p-tau area	-0.903	0.000024 ^a	-0.753	0.002
averaged β -amyloid area	-0.761	0.003	-0.651	0.012
APS	-0.761	0.003	-0.640	0.014

p-tau hyperphosphorylated tau; APS amyloid plaque score

^adenotes those comparisons below the Bonferroni-corrected p -value = 0.008



be inversely correlated with Prom1 methylation levels ($r_{\text{Spearman}} = -0.949$, $p = 0.051$) in the control group; meanwhile, no significant correlation was found between methylation and expression among AD patients ($r_{\text{Spearman}} = -0.287$, $p = 0.490$).

mRNA levels of *CRTC1* downstream target genes are altered in Alzheimer's disease hippocampus

We also wanted to test whether *CRTC1*-dependent genes were also dysregulated in our set of AD hippocampal samples. To this end, we measured mRNA levels of *BDNF*, *c-fos*, *EGR4*, and *EGR1* by RT-qPCR in our set of samples. Primer pairs and specific transcripts that were amplified by RT-qPCR are shown in Additional file 2: Table S2.

Interestingly, we found significantly decreased mRNA levels of *BDNF*, *EGR4*, and *EGR1* in AD hippocampus compared to control hippocampus (p -value < 0.05) (Additional file 1: Figure S4). These results are in accordance with previous reports based on AD in vitro models, murine models and human brain. On the contrary, *c-fos* mRNA expression was significantly

increased in AD cases compared to controls in our set of samples (p -value < 0.005) (Additional file 1: Figure S4).

Discussion

Here we report altered patterns of DNA methylation within two distinct promoter regions of *CRTC1* in the human hippocampus affected by Alzheimer's disease (AD). Interestingly, DNA methylation levels are decreased in AD and inversely correlate with p-tau burden in the hippocampus.

Our results indicate that important regulatory regions for the *CRTC1* gene are differentially methylated in the AD hippocampus compared to controls. It is well known that *CRTC1* is mainly expressed in the CA1 region of the hippocampus [8] and is essential to synaptic plasticity and memory consolidation [2–7]. Moreover, studies conducted on AD mouse models highlight its relevance to AD pathogenesis [3, 17–19] and *CRTC1* protein has been found to be downregulated in the human hippocampus affected by AD [19]. Thus, studying regulatory mechanisms of *CRTC1* gene expression in the human hippocampus, one of the more vulnerable regions to AD pathology, is of utmost interest. Our finding suggests

that altered patterns of methylation at regulatory regions might contribute to disruption of the *CRTC1* gene function that was previously described in AD [19], as DNA methylation is one of the most important epigenetic mechanisms that regulate gene expression. Most interestingly, methylation levels correlated to AD-related pathology changes in our cohort. In particular, a strong inverse correlation was found between DNA methylation levels and p-tau burden in the hippocampus ($r_{\text{Spearman}} = -0.903$, $p < 0.001$). It is of note that neurofibrillary tangles, which are aggregates of p-tau protein that accumulate within neurons, are found in the first stages of AD restricted to the hippocampus and entorhinal cortex, while the other hallmark of AD, β -amyloid deposition, begins to accumulate in the neocortex. From our data showing such a strong correlation, we may hypothesize that altered methylation within *CRTC1* may be part of the molecular signature of p-tau pathology in the hippocampus. Anyhow, the relationship between *CRTC1* gene and p-tau pathology merits further investigations.

We also found a mild but otherwise significant decrease in *CRTC1* mRNA expression in AD hippocampus compared to controls (Fig. 2). This finding is in concordance with a previous report that showed both total and phosphorylated CRTC1 protein downregulation in human hippocampus affected by AD [19]. Moreover, *CRTC1* mRNA expression has been found to be decreased in other neurodegenerative conditions and brain diseases, such as frontotemporal lobar degeneration with progranulin mutations [34] and Down syndrome [35] (Additional file 1: Figure S5). Down syndrome is considered a natural model for AD, since most people affected by Down syndrome will develop early AD [36]. Interestingly, a significant decrease in *CRTC1* mRNA levels was shown for *CRTC1* transcript variant 1 (NM_015321) but not for *CRTC1* transcript variant 3 (NM_001098482) in Down syndrome adult brain [35] (Additional file 1: Figure S5), which is in line with our present work.

To understand better the relationship between methylation and expression in our study we tested the correlation between *CRTC1* methylation and mRNA levels. Among control hippocampus samples, we found an inverse relationship between methylation and expression at the Prom1 region. In other words, lower levels of DNA methylation within Prom1 showed a trend to be correlated with higher levels of *CRTC1* mRNA ($r_{\text{Spearman}} = -0.949$, $p = 0.051$). This correlation is consistent with the classical model of epigenetic regulation where loss of methylation in the promoter region results in an increase in gene expression, indicating that *CRTC1* is probably regulated by DNA methylation under normal circumstances. However, no correlation between methylation and expression was

identified among AD patients in our analysis, suggesting that proper functioning of DNA methylation in regulating *CRTC1* expression may be disturbed in the AD context.

Finally, we show significantly decreased mRNA levels of several *CRTC1*-dependent genes, such as *BDNF*, *EGR4*, and *EGR1* in AD hippocampus compared to control hippocampus (p -value < 0.05) (Additional file 1: Figure S4), which adds consistency to the present study. These results are in accordance with previous reports performed on AD in vitro models, murine models and human brain. For instance, oligomeric $A\beta$ peptide downregulates *BDNF* levels via $A\beta$ -induced CREB transcriptional downregulation in human neuroblastoma (SH-SY5Y) cells [34]. In murine models, it has been largely demonstrated that several *CRTC1*-dependent genes are downregulated in the brain tissue affected by AD pathology, including *BDNF* and *EGR4* [17, 18, 35]. Most interestingly, not only are *BDNF* mRNA levels decreased in AD human frontal cortex but also *BDNF* mRNA levels correlate with cognitive performance of patients, assessed by Mini-Mental State Examination scores, as was shown in a recent report [36].

On the other hand, c-fos mRNA expression was significantly increased in AD cases compared to controls in our set of samples (p -value < 0.005) (Additional file 1: Figure S4). Remarkably, this is in good agreement with the results obtained from earlier studies that used AD human hippocampus to assess c-fos expression [37–39] but, in turn, differs from several reports that have shown c-fos mRNA levels to be decreased in AD murine models [17, 18, 35]. This discrepancy among the results obtained from murine and human studies regarding c-fos mRNA expression in the context of AD pathology will need further research.

Conclusions

In conclusion, this study provides evidence that *CRTC1* methylation levels are altered in the human hippocampus affected by AD. It also suggests that the functional relationship between DNA methylation and gene expression for *CRTC1* may be altered in the AD context. Further studies are needed to better understand the precise role that *CRTC1* methylation plays in AD pathophysiology.

Additional files

Additional file 1: Figure S1. Pictures obtained at 10x from the most representative cases showing different degrees of protein deposit. **Figure S2.** Beta-amyloid and tau protein measurement in hippocampal sections. **Figure S3.** Maps of the two *CRTC1* regions analyzed by bisulfite sequencing cloning in human hippocampus. **Figure S4.** mRNA expression levels of *CRTC1* downstream genes in human AD hippocampus compared to controls. **Figure S5.** Gene Expression Omnibus (GEO) data analysis for

CRTC1 mRNA expression levels in Down syndrome and Frontotemporal Lobar Degeneration with progranulin mutations. (PDF 974 kb)

Additional file 2: Supplemental Tables. **Table S1.** Brain sample set characteristics. **Table S2.** Bisulfite and quantitative-PCR primers. (PDF 308 kb)

Abbreviations

AD: Alzheimer's disease; APP: amyloid precursor protein; APS: amyloid plaque score; *BDNF*: Brain-derived Neurotrophic Factor; bp: base pair; cDNA: complementary DNA; ChIP-seq: chromatin immunoprecipitation sequencing; CpG: cytosine-phosphate-guanine dinucleotide; *CREB*: cAMP-responsive element binding; *CRTC1*: *CREB* regulated transcription coactivator 1; *EGR1*: Early growth response protein 1; *EGR4*: Early growth response protein 4; ENCODE: Encyclopedia of DNA Elements; GEO: Gene Expression Omnibus; HMM: Hidden Markov Model; mRNA: messenger RNA; PCR: polymerase chain reaction; Prom 1: promoter number 1; Prom2: promoter number 2; p-tau: hyperphosphorylated tau; RT-qPCR: real time-qPCR; UCSC: University of California, Santa Cruz.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MM conceived the study, participated in its design and coordination, obtained funds, analyzed data and contributed to writing/revising the manuscript. NC participated in designing parts of the study and contributed to drafting/revising the manuscript for content. MA participated in acquisition of data, revised subject diagnosis and contributed to drafting/revising the manuscript for content. JSR contributed to drafting/revising the manuscript for content, analysis and interpretation of data (p-tau and amyloid deposits), sorting of patients into different stages and acquisition and analysis of image data. MVZ participated in the study design, acquired data, revised subject diagnosis, classified patients, and contributed to drafting/revising the manuscript for content. MR contributed to design gene maps, performed bisulfite experiments, was involved in the interpretation of data, and revised the methods section of the manuscript. IR contributed to drafting/revising the manuscript for content and acquisition of data. RL contributed to acquisition of data, revised subject diagnosis and revised the draft. EE made substantial contribution to drafting/revising the manuscript for content with significant contribution to patients' staging and p-tau analysis. IM contributed to the study design, to obtaining funding and drafting/revising the manuscript for content. CE contributed to the study design, acquisition of data, sorting of samples, diagnosis of subjects and drafting/revising the manuscript for content. All authors read and approved the final manuscript.

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