

Research article

Expression of nucleotide excision repair in Alzheimer's disease is higher in brain tissue than in blood

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ABSTRACT

Age-related changes are increased in patients with Alzheimer's disease (AD), including oxidative stress and DNA damage. We propose that genotoxic stress and DNA repair responses influence neurodegeneration in the pathogenesis of AD. Here, we focus on nucleotide excision repair (NER). Real-time qPCR and mass spectrometry were employed to determine the expression levels of selected NER components. The mRNA levels of the genes encoding the NER proteins RAD23B, RPA1, ERCC1, PCNA and LIG3 as well as the NER-interacting base excision repair protein MPG in blood and brain tissue from four brain regions in patients with AD or mild cognitive impairment and healthy controls (HC), were assessed. NER mRNA levels were significantly higher in brain tissue than in blood. Further, LIG3 mRNA levels in the frontal cortex was higher in AD versus HC, while mRNA levels of MPG and LIG3 in entorhinal cortex and RPA1 in the cerebellum were lower in AD versus HC. In blood, RPA1 and ERCC1 mRNA levels were lower in AD patients than in HC. Alterations in gene expression of NER components between brain regions were associated with AD, connecting DNA repair to AD pathogenesis and suggesting a distinct role for NER in the brain.

1. Introduction

Although Alzheimer's disease (AD) has been recognized since the beginning of the 20th century, we still have little information about the etiology at the molecular level. One of the main challenges in AD research is to define biomarkers with high sensitivity and specificity that are present in the pre-symptomatic stages of the disease, so that intervention can be initiated at an early stage. An optimal test would be a set of biomarkers present in a blood sample. However, in order to achieve this, we need more knowledge on the etiology of early AD.

Aging is the major risk factor for the development of AD, and age-related changes are increased in AD and mild cognitive impairment (MCI), including DNA damage and oxidative stress [2,3,5,10,16,25,26]. We propose that an imbalance in oxidative stress and DNA repair responses influence neurodegeneration in AD. DNA damage is repaired by several DNA repair mechanisms, including nucleotide excision repair

(NER) and base excision repair (BER).

The NER pathway repairs bulky helix-distorting damage events in DNA, which can be caused by several various sources including UV radiation, chemical adducts and oxidative stress. NER is one of the most well-characterized mechanisms of DNA repair, and defects in the NER pathway are recognized causes of several neurodegenerative diseases, such as Cockayne syndrome (CS), trichothiodystrophy and some subtypes of xeroderma pigmentosum (XP) [6,17]. The association between NER and other neurodegenerative disorders in humans is, however, poorly understood [21]. It is proposed that NER-inflicted neurodegeneration most likely is caused by endogenous DNA lesions [1], since exogenous stress such as UV radiation does not directly affect the brain and most chemical adducts do not cross the blood-brain barrier. Endogenous DNA lesions are most commonly caused by reactive oxygen species (ROS) and as a consequence, damaged macromolecules including DNA are generated.

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The NER pathway involves more than 30 enzymes and is separated into two different pathways based on the manner of damage recognition [11]. The transcription coupled repair pathway (TC-NER) repairs DNA lesions that are detected through the blockade of the RNA polymerase II enzyme, while in global genome (GG)-NER, the damage is mostly recognized by the XPC-RAD23B complex [12,21]. RAD23B has been shown to interact with the 3-methyladenine DNA glycosylase (MPG) of the BER-pathway and elevates glycosylation of MPG-specific DNA damages [14], suggesting its effect in damage recognition in both NER and BER.

The remaining part of the NER repair pathway is common for both sub-pathways. The damaged DNA helix is unwound by helicases in the transcription factor II H-complex (TFIIH). The Replication Protein A (RPA)-complex, with the subunits RPA1-3, binds to the undamaged DNA strand. Incisions in the damaged DNA strand are made by XPG and the XPF-ERCC1-complex, respectively, in the 3' and 5' sites of the damage event. This results in the release of an oligomer of 27–30 nucleotides [21]. The remaining gap is filled by the DNA polymerase subunits δ , κ and ϵ , which are recruited by the PCNA clamp, and is finally sealed by either LIG1 or the XRCC1-LIG3-complex [11,21].

The BER pathway is the predominant DNA repair pathway for the processing of small base lesions derived from oxidation and alkylation events [20]. Multiple studies show altered BER profiles in the prodromal phases of AD in both mice [9,15,22] and human brain tissue [8,10,25]. Less is, however, known about NER in human neurodegenerative disorders [21]. Although mice lacking NER components such as ERCC1, XPF and other enzymes related to XP or CS have provided good models for neurodegeneration [17], their potential role in explaining the etiology of AD still remains unclear.

2. Materials and methods

2.1. Ethical statement

The study was approved by the Norwegian Regional Committee for Ethics in Medical Research (REK 2013/1643 and REK 2011/698). Written, informed consent was obtained from all participants. Donation, storage and transfer of human brain specimens were also approved by the external Research Ethical Committee of the Fundacion Centro de Investigación de Enfermedades Neurológicas (CIEN) biobank (Research Ethical and Animal Welfare Committee, ISCIII, Spain).

2.2. Brain specimens and blood samples

Freshly frozen post-mortem brain tissue specimens from a cohort of 43 AD patients and 9 healthy controls (HC) were harvested by CIEN. Specimens from the frontal cortex (FC), cerebellum (CB), entorhinal cortex (EC) and the hippocampus (HCP) were investigated, representing a total of 157 brain specimens from 43 AD patients and 33 brain specimens from 9 HC (Table S1). For further details, see Additional file 1 in Lillenes et al. [8].

Blood samples from 51 AD patients, 24 MCI patients and 62 HC were collected in PAXgene tubes at the Memory Clinic at Oslo University Hospital (Ullevål) [23]. AD patients were diagnosed to have suspected AD according to the NINCDS-ADRDA criteria [13], while patients with MCI had to fulfill either the ICD-10 criteria or the Winblad criteria for MCI [27]. Patients with frontotemporal, Lewy-Body and vascular dementia, as well as those with severe depression or psychotic features, were excluded from the study. All samples were immediately stored at -80°C until further use.

2.3. RNA isolation

For information on RNA isolation and determination of RNA concentration, please see the Supplementary Material.

2.4. Quantitative real-time PCR

Reverse transcription and quantitative real-time PCR (qRT-PCR) was performed using TaqMan Gene Expression assays for the RAD23B (HR23B), RPA1, ERCC1, PCNA, LIG3, and MPG mRNAs (Table S2). Glyceraldehyde phosphate dehydrogenase (GAPDH) was selected as the reference gene after validating 32 candidate genes as internal control for all four brain regions and blood samples in both AD and HC, using TaqMan Human Endogenous Control Plates (Applied Biosystems, Foster City, California, USA). For further details, see the Supplementary Material and Additional file 1 in Lillenes et al., 2016 [8].

2.5. Proteomic analysis by mass spectrometry

Proteomic analysis was performed using high-resolution mass spectrometry (Q-Exactive, Thermo-Fisher). For further information regarding protein sample preparation and analysis, please see the Supplementary Material.

2.6. Statistical analysis

To statistically test the differences of mRNA levels in blood and brain tissue, a two-sample *t*-test was performed (Table 1, S3, S4). The level of significance was adjusted using the Bonferroni correction.

The mRNA levels in blood in the AD, MCI and HC groups were statistically compared using a one-way ANOVA analysis (Table 2). If the *F*-test *p*-value was less than 0.05, Tukey's test was used for pairwise comparisons which controls the family-wise error rate [24]. A linear mixed model was used to test the differential mRNA levels between AD and HC in the four human brain regions for each of the six genes (Table 3). The fixed effects in the model were disease, brain region and the interaction between disease and brain region. Potential correlation between brain regions from the same patient was corrected for using a random individual effect. An *F*-test was used to monitor the significance of the overall interaction between brain part and disease status. When the overall interaction effect was non-significant, the significance of the disease effect could be assessed directly using a *t*-test. If the interaction effect was significant, a reduced mixed model was used to test the interactions separately for each brain part. This model included fixed effects for brain region, interaction between brain region and disease status and the random effect. To test the interaction of disease status and brain part, a *t*-test was used with a significance level of 0.05.

3. Results

3.1. Higher NER mRNA levels in brain tissue than in blood

We compared mRNA levels from brain tissue and blood samples from AD patients and HC (Table 1). mRNA levels for RAD23B, RPA1, ERCC1, PCNA, LIG3 and MPG were monitored (Fig. 1). The most

Table 1
mRNA levels of RAD23B, LIG3, ERCC1, RPA1, PCNA and MPG in brain and blood.

Gene	Mean GE in blood	Mean GE in brain	p-value	Mean GE		p-value
				in blood	GE in brain	
	Alzheimer's disease			Healthy controls		
RAD23B	0,80	1,26	1,77E-09 *	0,88	1,27	6,00E-03
RPA1	0,73	1,54	< 2,2E-16 *	0,88	1,69	8,00E-04 *
ERCC1	0,58	2,42	< 2,2E-16 *	0,67	2,04	7,70E-06 *
PCNA	0,85	1,50	9,00E-08 *	0,94	1,24	7,10E-02
LIG3	0,48	2,58	< 2,2E-16 *	0,51	2,44	1,30E-07 *
MPG	0,92	1,20	6,90E-05 *	1,01	1,27	4,00E-02

*denotes significant results after Bonferroni-correction.

Table 2
Comparison of mRNA levels between blood samples of AD and HC.

Gene	AD blood mean	MCI blood mean	HC blood mean	ANOVA p-value	AD vs. MCI, p-value	MCI vs. HC, p-value	AD vs. HC p-value
RAD23B	0,8	0,79	0,88	0,15			
RPA1	0,73	0,78	0,88	0,02*	0,72	0,32	0,01*
ERCC1	0,58	0,59	0,67	0,02*	0,96	0,15	0,02*
PCNA	0,85	0,92	0,94	0,37			
LIG3	0,48	0,53	0,51	0,35			
MPG	0,92	1,06	1,01	0,24			

*denotes significant results. The ANOVA p-value is the p-value of ANOVA F-test and the p-values in the three last columns are from Tukey's test.

Table 3
mRNA levels of RAD23B, LIG3, ERCC1, RPA1, PCNA and MPG in brain regions of AD and HC.

DNA repair enzyme	Overall interaction effect p-value	AD Effect	p-value	Specific interactions Brain region	Effect	p-value
RAD23B	0,3095	0,968	0,3343	↓	–	–
RPA1	0,0006	–	–*	↓	–	–
				Frontal cortex	–0,605	0,5459
				Cerebellum	3739	0,0002*
				Hippocampus	–0,726	0,4688
				Entorhinal cortex	–0,201	0,8409
ERCC1	0,1171	–1865	0,0638	↓	–	–
PCNA	0,6662	–0,092	0,9268	↓	–	–
LIG3	0,0006	–	–*	↓	–	–
				Frontal cortex	–2879	0,0045*
				Cerebellum	1087	0,2784
				Hippocampus	–1457	0,1468
				Entorhinal cortex	2251	0,0256*
MPG	0,0411	–	–*	↓	–	–
				Frontal cortex	0,031	0,9753
				Cerebellum	0,25	0,8029
				Hippocampus	–1217	0,2252
				Entorhinal cortex	2313	0,0218*

The p-value in the AD column is the result of the t-test comparison of AD vs. HC, which was applied when the overall interaction effect (second column) was not significant. If the overall interaction effect was significant, the p-values for the specific interactions of brain region and AD/HC group were calculated (last column). * denotes significant results, where the p-value is below 0.05.

prominent differences were detected between brain tissue and blood samples in the AD patients. When comparing AD brain tissue with AD blood samples, there was significantly higher levels of mRNA of all the six genes in the brain tissue compared to blood (Fig. 1A). When comparing brain tissue and blood in HC, the overall mRNA levels were significantly higher in brain for RPA1, ERCC1 and LIG3 (Fig. 1B). Although higher mRNA levels for RAD23B, PCNA and MPG were detected in brain compared to blood, these differences were not significant.

3.2. Differential NER mRNA levels in blood samples from AD and HC

When comparing mRNA levels in blood samples from AD patients and HC, mRNA levels of RPA1 (p: 0.01) and ERCC1 (p: 0.02) were lower in blood samples from AD patients (Fig. 2). The mRNA levels for the genes encoding RAD23B, PCNA, LIG3 and MPG were similar (Table 2, Fig. 2). Overall, the mRNA levels in blood tended to be lower in AD than in HC. There was no significant difference in mRNA levels between neither MCI and AD nor MCI and HC.

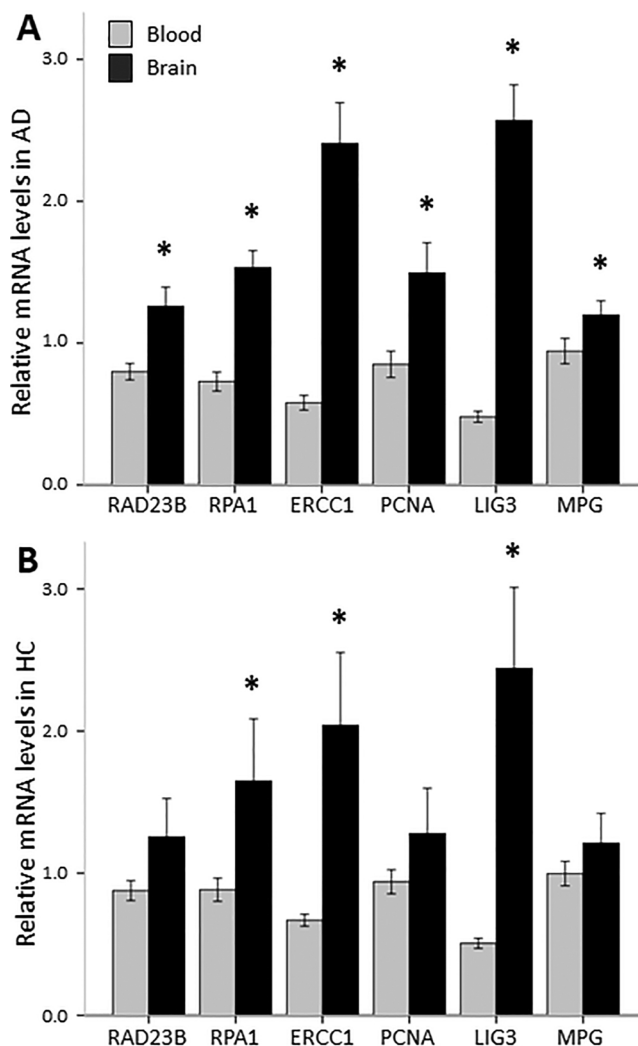


Fig. 1. mRNA levels of RAD23B, RPA1, ERCC1, PCNA, LIG3 and MPG compared between human blood samples and brain tissue (mean ± 2SEM). * denotes significant differences corrected for with the Bonferroni correction. A) Comparison of mRNA levels between blood samples and brain tissue in AD patients. B) Comparison of mRNA levels between blood samples and brain tissue in HC.

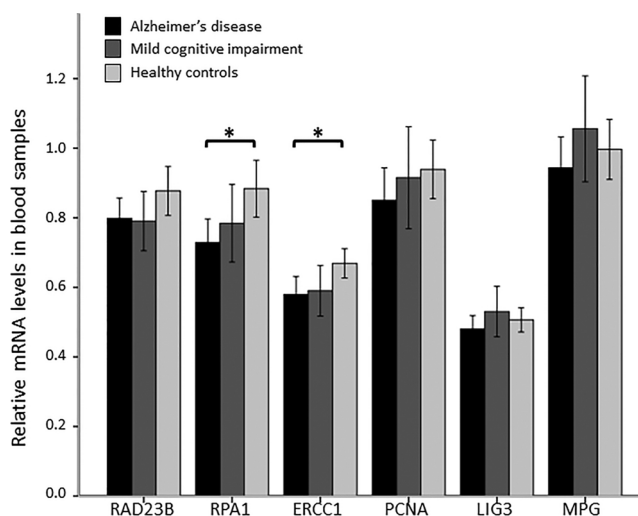


Fig. 2. Comparison of mRNA levels in blood samples from AD and MCI patients and HC (mean ± 2SEM). * denotes significant difference with a p-value below 0.05, based on a F-test in an one-way ANOVA and paired comparison with Tukey's test to control for family wise error rate.

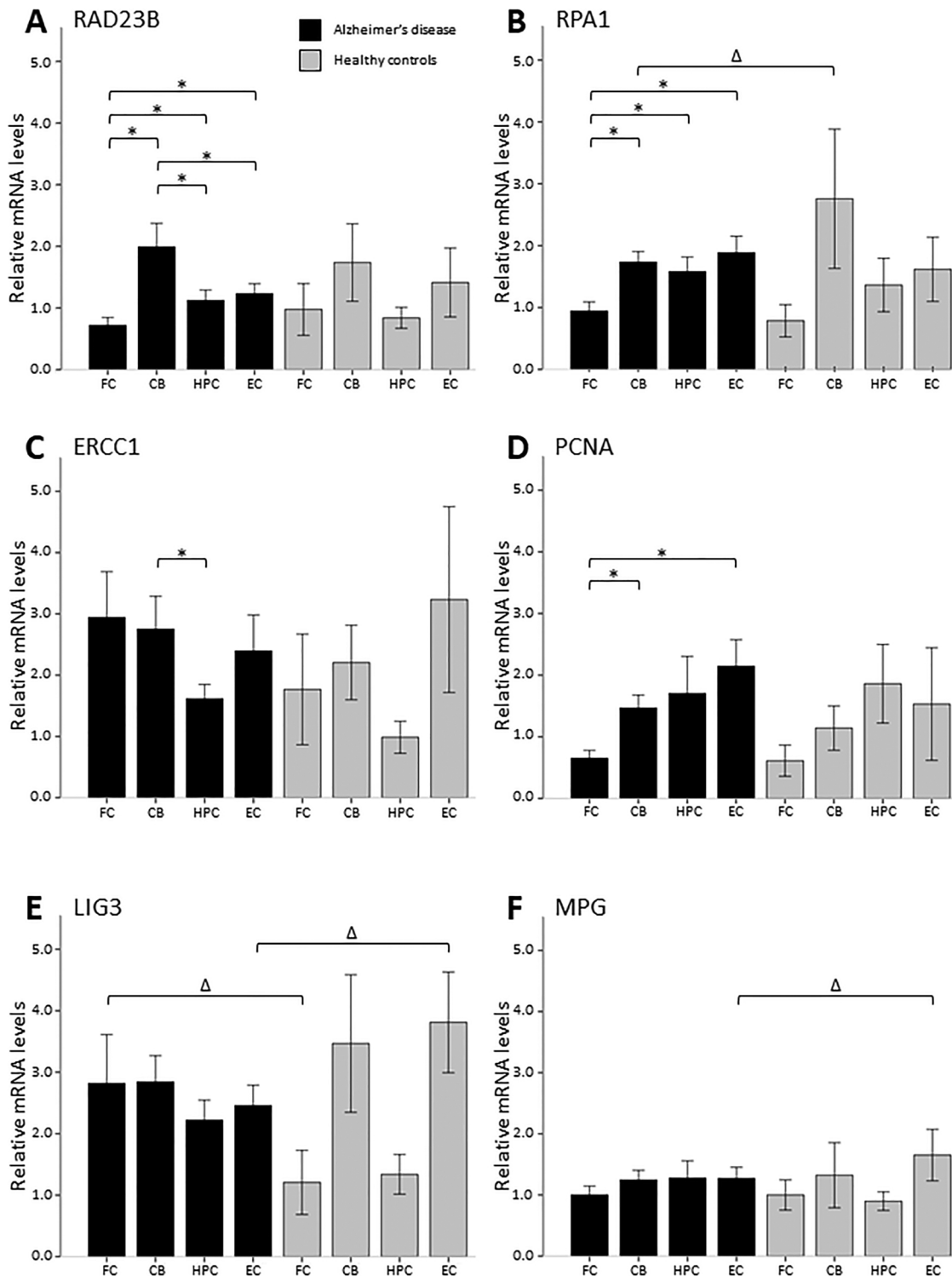


Fig. 3. RAD23B, RPA1, ERCC1, PCNA, LIG3 and MPG mRNA levels in brain regions of AD patients and HC (mean ± 2SEM). * denotes significant p-value < 0.00069 based on Bonferroni correction with 72 tests. Δ denotes significant difference in mRNA levels between AD and HC (see Table 3). A) Comparison of RAD23B mRNA levels in blood and brain tissue. B) Comparison of RPA1 mRNA levels in blood and brain tissue. C) Comparison of ERCC1 mRNA levels in blood and brain tissue. D) Comparison of PCNA mRNA levels in blood and brain tissue. E) Comparison of LIG3 mRNA levels in blood and brain tissue. F) Comparison of MPG mRNA levels in blood and brain tissue.

3.3. Differential brain mRNA levels between AD and HC

mRNA levels of RPA1 was significantly lower in the cerebellum in AD as compared to HC (Table 3, Fig. 3). The mRNA levels of LIG3 were found to be significantly higher in the frontal cortex of AD patients, and significantly lower in the entorhinal cortex as compared to HC. The MPG mRNA levels were significantly lower in the entorhinal cortex of

AD patients than in HC. There was no significant difference between AD and HC in the mRNA levels for RAD23B, ERCC1 or PCNA.

3.4. Differential mRNA levels between brain parts in AD and HC

mRNA levels of RAD23B were significantly higher in the cerebellum as compared to the frontal cortex, hippocampus and entorhinal cortex

in AD patients (Table 4S, Fig. 3A). Furthermore, the mRNA levels of RAD23B in the frontal cortex were significantly lower compared to the hippocampus and entorhinal cortex. mRNA levels of RPA1 in AD were significantly lower in the frontal cortex as compared to the cerebellum, hippocampus and entorhinal cortex (Table S4, Fig. 3B). mRNA levels of ERCC1 were significantly lower in the hippocampus compared to the cerebellum in AD (Table S4, Fig. 3C). The mRNA levels of PCNA in AD were significantly lower in the frontal cortex compared to the cerebellum and entorhinal cortex (Table S4, Fig. 3D). There were no significant differences in mRNA levels for LIG3 or MPG, neither in AD nor HC (Table S4, Fig. 3E–F). Relatively low levels of brain MPG mRNA was detected compared to RAD23B, RPA1, ERCC1, PCNA and LIG3 mRNAs.

In HC, there were no significant differences in mRNA levels between the brain regions. Although not significant, the RAD23B mRNA levels tended to be highest in the cerebellum among HC as in AD. Similar PCNA and MPG mRNA levels were found in AD and HC.

3.5. mRNA levels in different brain regions varied compared to blood

When each brain part was analyzed separately, we found significantly higher mRNA levels of LIG3 and ERCC1 in all the four brain regions in AD patients, as compared to blood (Table S3). For RAD23B and RPA1, all brain regions except the frontal cortex had significantly higher mRNA levels compared to blood. For PCNA, there were significantly higher mRNA levels in the brain compared to blood for cerebellum and entorhinal cortex, while the mRNA levels for MPG were only significantly higher in cerebellum than in blood. In the comparison between each brain region and the blood samples of HC, the only significant difference was found for LIG3, showing higher mRNA levels in the cerebellum and entorhinal cortex compared to blood.

3.6. Proteomic detection of RAD23B, RPA1 and MPG in brain tissue

Next generation mass spectrometry (MS) was performed on 13 brain tissue specimens from the frontal cortex and cerebellum from both AD and HC. Relatively high protein levels of RAD23B and RPA1 were detected in all samples. MPG was detected in one cerebellum sample from HC (Fig. S1), while the other NER proteins were below the detection level. There was, however, no statistically significant difference between protein levels in AD and HC.

4. Discussion

The aim of this study was to investigate the expression of selected NER enzymes in human brain tissue and blood, to detail their presence in AD and HC physiology and to search for potential biomarkers for early AD development. Notably, the mRNA levels of RAD23B, RPA1, ERCC1, PCNA, LIG3 and MPG were significantly higher in brain tissue than in blood samples in AD patients.

The vast amounts of energy required by the brain, warrants that this tissue is highly vascularized. Circulating blood cells respond to changes in the macro- and micro-environment, and express specific signatures in their responses to both physiological and pathological changes in the body [7]. Despite the limitations of direct interactions of blood with the brain through the endothelium and the blood-brain-barrier, blood samples can be a useful surrogate in reflecting the current brain status, as brain tissue is generally not available from living human individuals. Higher levels of all NER components investigated in brain compared to blood underscored the importance of their function in NER-derived DNA repair in the brain. The high mRNA levels of LIG3, PCNA and MPG signify the important function of BER-derived DNA repair in the human brain, corroborating former BER studies [8,10]. Higher levels of both NER and BER mRNAs in the brain compared to blood also demonstrated that the integrity of the post-mortem brain tissue was intact. Due to immediate post-mortem degradation of RNA in general, these brain mRNA levels might have been even higher.

Lower mRNA levels of RPA1 and ERCC1 were observed in the blood samples of AD patients compared to HC. These findings suggest that decreased DNA repair of replication by the RPA-complex and decreased endonuclease function of the XPF-ERCC1-complex potentially could be a part of the pathogenesis of AD. RPA1/ERCC1 mRNA levels should therefore be investigated as a potential AD biomarker, making them interesting topics for future studies. However, no significant differences for RPA1 and ERCC1 mRNA levels were detected in MCI. MCI is considered to be the prodromal stage of AD. However, not all patients with MCI develop AD, and some patients with MCI may even recover [19]. This heterogeneity could explain the high standard deviation (SD) found in the collective MCI group and why significant differences were absent for MCI, precluding the identification of a pre-AD blood biomarker in this study.

Differential mRNA levels for RPA1, LIG3 and MPG in specific brain regions between AD and HC suggest that dysregulation of DNA repair responses is an underlying effect in the pathophysiology of AD. The higher LIG3 mRNA levels in the frontal cortex and the lower mRNA levels of MPG and LIG3 in the entorhinal cortex and RPA1 in the cerebellum in AD versus HC may reflect the predominant temporal lobe affection in AD. However, the combination of brain region specificity and NER mRNA levels make these findings somewhat difficult to interpret.

The SDs of mRNA levels between brain regions varied more in HC than in AD. The increased SD in HC can be explained by the small sample size, but could also reflect the greater potential for adaptation and compensation among the DNA repair components in the healthy brain. The reduced variation among brain regions in AD can be a result of low brain heterogeneity in the late final stages of AD, as these samples are based on post-mortem brain specimens. In AD, the mRNA level of RAD23B in cerebellum was significantly higher compared to all the other brain regions. Cerebellum is the last brain region to be affected in AD, making RAD23B activity an interesting target for further studies of the early pathogenesis in AD.

The lack of detection of PCNA, ERCC1 and LIG3 protein can be explained by the low abundance of DNA repair components in general, in combination with the limited sensitivity of the MS analysis. The low sample size reduces the power to identify differences in protein abundance and could explain the lack of significant differences for RAD23B and RPA1.

The optimal approach for this study would be to investigate blood and brain tissue from the same patients. This was, however, challenging as blood could be drawn from living patients and participants, securing non-degraded mRNA, while the brain tissue for ethical reasons had to be harvested post-mortem.

AD is still most prevalent in the industrialized parts of the world, with its high standard of living. This is mostly due to the long life-time expectancy as aging is the greatest risk factor in development of AD. However, different systems for AD diagnostics between countries and world regions may also influence these statistics. The World Alzheimer Report of 2015 stated a high prevalence of AD in Western Europe, although there was no clear pattern between the different countries [18]. In their meta-analysis, where Italy was the reference country (due to the highest number of studies), Norway (1.04) had a similar prevalence-ratio to that of Spain (1.02) [18]. The usage of two different cohorts from these countries may therefore not represent a major source of error, although local life style, cultural differences and genetic variations must be taken into account in any between-country comparison. Genes associated with increased risk of developing AD, such as the $\epsilon 4$ allele of Apolipoprotein E (APOE $\epsilon 4$), show differences in allele frequency in some population cohorts in the Scandinavian and Mediterranean countries of Europe. However, the allele frequencies for these genes were found to be rather similar in both Norway and Spain [4].

The gender distribution in both cohorts was adequately balanced. Due to the many *t*-tests performed in this analysis, the Bonferroni correction was applied to address the challenge of multifactorial testing.

5. Conclusions

Higher levels of NER and BER mRNAs in the brain compared to blood underscore the important role of DNA repair in the brain. Expression of RPA1 and ERCC1 in blood may potentially be investigated in the future search for biomarkers for pre-symptomatic AD. Different expression of RPA1, LIG3 and MPG in various brain regions between AD and HC connects these components to the pathophysiology of AD.

Contributions

Conceived and designed the experiments: T.T. and M.S.L. Provided materials: A.R., I.D.U., and T.B. Performed research: H.L.B.J., M.S.L., T.R., S.T.K. and T.T. Analyzed data: H.L.B.J., M.S.L., T.R., S.T.K. and T.T. Statistical analysis: C.G. Wrote the paper: H.L.B.J., M.S.L. and T.T. All authors have approved the final article.

Conflict of interest

The authors have no conflict of interest to report.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.neulet.2018.02.043>.

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