UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Impact of strong psychologically stressful events on the development of Alzheimer disease: a possible role of epigenetic?

Students
Sarah El-Achachi
Fabio Schipani

Tutor
Prof. J. Ghika
Leenaards Memory Center, Department of Neurosciences, CHUV

Co-tutor
Prof. J.F Démonet
Leenards Memory Center, Department of Clinical Neurosciences, CHUV

Expert
Dr. F. Assal, PD, Service de Neurologie, Hôpitaux Universitaires de Genève
HCUGE

Lausanne, December 15th, 2014

Impact of strong psychologically stressful events on the development of Alzheimer disease; what is the role of epigenetic?

Sarah El-Achachi[1]*, Fabio Schipani[1], Joseph Ghika[2]

Abstract
Alzheimer disease (AD) is the most common neurodegenerative dementia. It leads to a progressive loss of cognitive functions, especially memory. Most of AD cases are sporadic, resulting from the interplay of genetic and environmental factors which get involved in the regulation of expression of thousands of genes, a mechanism called epigenetic. Epigenetic modifications, by modifying genes transcription, help to orchestrate the phenotypical changes linked to development, aging or even diseases and cancer. In AD, recent studies showed rapid, dynamic and persistent epigenetic mutations that are believed to have consequences on brain functions. One of the earliest biomarker of AD is amyloïd-beta (Aβ) deposition in the brain. According to current studies, deposition of amyloïd-beta begins approximately 20 years before the first symptoms linked to the disease which questions us about what could have happened around or before that time. In this exploratory study, we searched if there could be any correlation between the experience of a strong psychologically stressful event in life, which could have lead to several epigenetic changes and therefore the occurrence of Mild Cognitive Impairment (MCI) or AD approximately 30 years later, and to see if there is a difference in the delay between amnestic MCI and AD patients.

Keywords
Alzheimer disease — Epigenetics — Traumatic life events

[1] *Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland*
[2] *Professor at the Leenaards Memory Centre, CHUV, Lausanne, Switzerland*
***Corresponding author**:sarah.el.achachi@gmail.com

Contents

Introduction

Alzheimer's disease (AD) is the leading cause of dementia, and the most prevalent neurodegenerative disease in the elderly. Demographic changes and the current absence of cure for the disease cause the number of patients afflicted by dementia worldwide to increase from 35.6 million in 2010 to 115.4 million in 2050 or even more according to recent studies [1, 2]. AD is a progressive, irreversible disorder clinically characterized by a progressive impairment in memory and other cognitive abilities. At the microscopic level, the characteristic lesions in Alzheimer's disease are senile plaques, due to amyloïd-beta protein (Aβ) aggregation and neurofibrillary tangles due to tau hyperphosphorylation, together with a degeneration of the neurons and synapses due to neurovascular dysfunction, and other mechanisms such as cell-cycle abnormalities, inflammatory processes, oxidative stress, and mitochondrial dysfunction, leading to massive neuronal loss, mainly in the hippocampus and other associative regions of the neocortex [3]. The studies of these brain changes and other biological markers helped to know more about the chronological sequences of AD, using brain Aβ amyloidosis (evaluated by positron emission tomography (amyloid PET imaging), elevated tau and phosphorylated tau and decreased Aβ in the cerebrospinal fluid (CSF), decreased fluorodeoxyglucose 18F (FDG) uptake on PET-scan with a medial temporal, posterior cingulate and temporoparietal pattern of hypometabolism, and brain atrophy on structural quantitative magnetic resonance imaging (VBM voxel based morphometry MRI). All of them are now used as biomarker of AD related changes,

as shown in Fig. 1. Biomarkers are also contributing to the clinical classification of the different phases of the disease according to the revised criteria of 2011 [4] which also give a chronological scale to the disease advances, as shown in Fig. 2. Although the neuropathological changes associated with AD have been well characterized, there is still considerable debate regarding the underlying etiology of the disorder and the precise mechanism(s) that prevail behind disease progression. A genetic etiology has been found especially in early onset AD (<65 years old). Autosomal dominant mutations in 3 genes (APP, PSEN1, and PEN2) are incriminated but these genetic mutations account for only 5%–10% of the total disease burden. Most cases of AD are at late onset (65 years), non-Mendelian, and highly sporadic [5]. A genetic risk for late onset AD remains ; the $\varepsilon 4$ allele coding the apolipoprotein E (APOE), this allele increases the risk of the disease by 3 times in heterozygotes and by 15 times in homozygotes. It is thought to be one of the two main risk factors for AD with increasing age [3]. Other susceptibility genes have been reported by genome-wide association study (GWAS). They all add quantitatively to an individual's personal risk. Beside genetic etiologies, emerging evidence from animal and human studies suggests that specific transcriptional mechanisms including DNA methylation, histones modifications and chromatin remodeling, and non-coding RNA (ncRNA) regulation, which determine how and when genes are expressed without altering the genetic code, are contributing to AD. These mechanisms are known as epigenetic [6]. Studies on the expression of memory-permissive genes regulation mechanisms showed that epigenetic mechanisms are dynamic and reversible, suggesting that the epigenome can be quickly altered in response to experiential stimuli and that this epigenome can stably persist throughout the lifespan [7, 8]. This information find its interest in the fact that as epigenetic changes affect genes transcription mechanisms, their effects can give rise to different cell types and developmental stages, and be either favorable, unfavorable or neutral for specific cells within the same organ system that can have an impact on the life of an individual. For example, increasing the accessibility of a gene regulatory element could either increase or decrease transcription of nearby genes depending on whether an activator or a repressor binds at that site. We can easily understand then how complex research in that field becomes, especially now that it is known that epigenetic changes can be induced by different environmental, or simply stochastic events, as it is showed by an increasing number of studies in many different medical fields (Millington, 2008: UV and cutaneous malignancies; Zannas and West, 2014: psychiatric disorders; etc.) [9, 10]. Specific studies about learning and memory confirmed (in vitro) that there is a functional role of DNA methylation and histones acetylation in spontaneous neurotransmission and short-term synaptic plasticity in the acquisition and persistence of altered behavioral responses of the organism to an environmental stimulus [11]. Moreover, it has been shown that dynamic regulation of gene expression by epigenetic mechanisms is a necessary and critical process in the formation, storage and recall of memory and subsequent behavior. Indeed DNA methylation and phosphorylation changes are essential for the formation of LTM (long term memory), consolidation and recall memory. Likewise, there is growing evidence showing that histones acetylation have also effects on memory and behavior, especially LTM [12]. These changes are observed in the hippocampus, amygdala and cortex in response to learning which are also the anatomical regions involved in AD pathology [7, 13, 14, 15]. Such discoveries about epigenetic outcomes and memory regulation speak for a probable link between epigenetic changes and AD and underline the difficulty to link a specific mechanism to a specific outcome.

One possible etiology of epigenetic changes and memory function is mental stress. As memories are built on the environment, they play a crucial part of how we adapt to changes in the environment thereby preparing us cognitively, emotionally, and physiologically to all kind of life events. We know that we make particularly strong memories of traumatically stressful events in our lives, therefore, strong psychologically stressful events are crucial in their impact on survival and inclined to have a long-lasting impact on behavior [16, 17]. The behavioral responses to stressful events involves changes in gene expression in limbic and paralimbic brain regions such as the hippocampus and amygdala and cingulate gyrus, once again, the brain regions that are more fragile in AD. A vicious circle exists between early-life adversity, hypothalamic-pituitary-adrenal axis regulation - therefore stress response - and future chronic stress, which acts through epigenetic mechanisms to reprogram expression patterns of the glucocorticoïd receptors, therefore influencing the sensitivity of an individual's response to environmental stressors. In brief, life experience modifies the epigenome, and as epigenome is related to memory-related gene expression, it means that experience through life events are critical for the plasticity that occurs in response to learning and memory [7]. Following what we know about chronological brain changes in AD and the strong impact of psychologically stressful events on the epigenome, we worked on an exploratory qualitative study, aiming to determine if there is a correlation between the experience of a traumatic life event and the occurrence of amnestic MCI and AD, or if it exists a restricted critical time interval between the event and the onset of symptoms. In a more clinical goal we will try to give arguments on the interest of taking the past psychological life events history of patient coming with memory complaint. Could this be of future use for a better approach and understanding of the environmental factors in the beginning of MCI and AD disorders?

1. Methods

1.1 Subjects

Our qualitative study is based on 45 telephonic interviews. 30 patients were recruited between 2013 and 2014 from the "Leenaards Memory Center of the CHUV, Lausanne", accord-

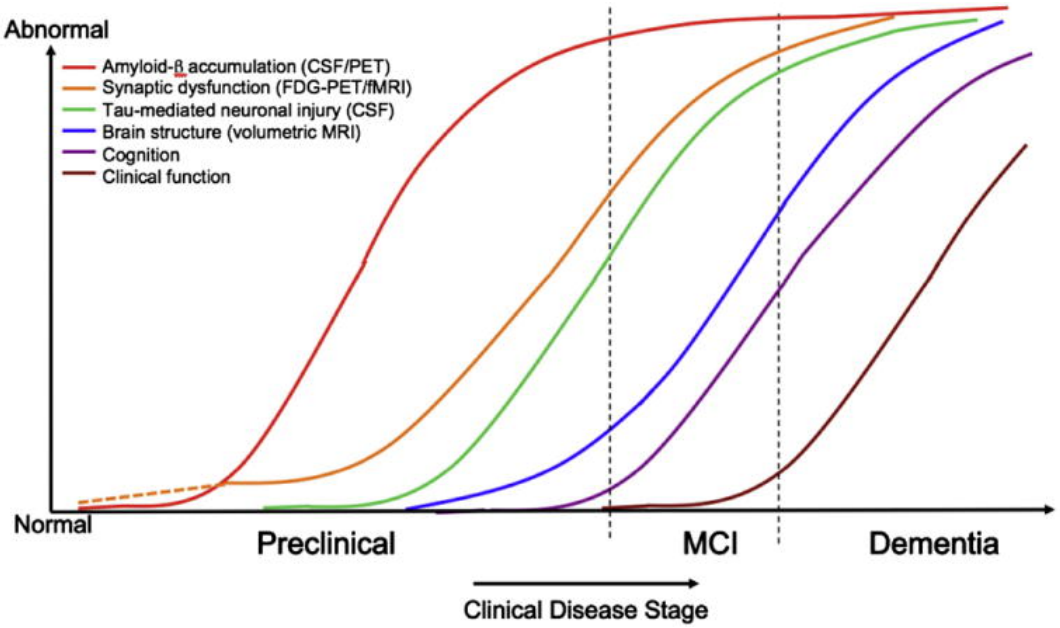

Figure 1: Hypothetical model of the dynamic of biomarkers in AD development [18].

ing to the revised criteria of 2011 for diagnosis of AD [4]. 15 patients with known incipient Alzheimer dementia (clinical dementia rating, CDR of 0.5-1) were selected, 15 with amnestic MCI (executive dysfunction allowed), and 15 control cases, with no complaint nor sign of cognitive impairment or psychiatric disorder. All the subjects were older than 50 years, and MMS score was available for the 30 patients recruited at the "Leenaards Memory Center". Familial cases were rejected in order to focus on patients with a sporadic form of AD.

1.2 Telephonic interviews

Our questionnaire can be found in the appendix section of this paper. The purpose of this questionnaire was to investigate the occurrence of traumatic life events in patients and control cases through one open question (the interviewed person recall spontaneously the traumatic event) and the following proposed list of traumatic events:

- serious accident or wound
- mental or physical violence
- sexual abuse
- painful mourning
- divorce/separation/infidelity
- being witness of a catastrophe
- facing a difficult diagnosis
- difficult treatment/operation/hospitalization
- death Note
- missing person
- serious financial problem
- trouble with justice
- dismissal/unemployment/professional threat
- deportation
- psychiatric disorder
- other not mentioned

The primary idea was to focus on traumatic events that occurred around 20 years before the first symptoms of the disease, as it has been shown that it is the approximate lapse of time between the first appearance of pathological changes of AD and these first symptoms [18], but due to the difficulty to recall the exact year of the events, we choose to split the events into two categories: events that happened more than 30 years and events that happened less than 30 years before today (2014). The dates of symptoms onset were found in patients medical records. The ethical comity did not show any concern regarding our study, as all the participants gave their enlighten consent to participate to the interviews which were made only because these items were insufficiently reported either in details or even at all mentioned in previous summaries of the personal and social history of their charts. These interviews are considered as "further patient history". If we can prove that history of traumatic life-events can be of any help for treatment or early diagnosis of AD, then this kind of interview could become a part of patients medical folder for more analysis.

1.3 Data analysis

If we know the approximate time of pathological changes (Aβ deposition and tau hyperphosphorylation), approximately 20 years before the first symptoms of AD, it is more difficult to find in the literature any strong timing correlation between epigenetic changes and the onset of the disease. Indeed, studies showed that epigenetic changes following stress does not affect the accumulation of Aβ nor tau [20]. It is also known that some epigenetic changes that happened in early childhood are creating a predisposition to certain diseases, such as depres-

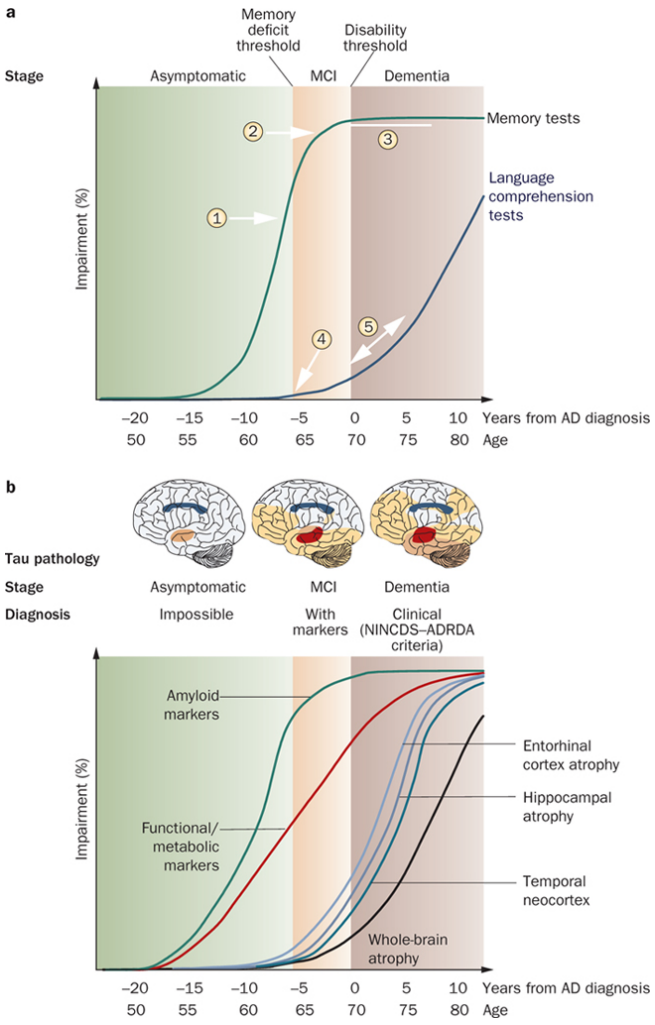

Figure 2: Theoretical model of cognitive and biological markers progression in AD. In this model, we can see that there is approximatively 5 years between the first memory symptoms, marking the beginning of MCI phase, and the development of dementia [19].

sion or cancer, later on. This is the reason why we chose to extend the investigation of traumatic life-events to the whole patient life, and separated the data into two categories, "events that happened more than 30 years from now" and "events that happened less than 30 years from now". Knowing the year of symptoms onset in MCI and AD patients and the date when the traumatic event happened, we calculated another variable that we called "latency". "Short latency" encompasses traumatic events that happened after 1984, as "Long latency" encompasses traumatic events that happened before 1984.

1.4 Statistical analysis

All analyses were performed using STATA. Statistics have been mostly performed on data coming from the two groups of persons having a known MMS score (MCI and AD patients), which means all the statistical analysis found in tables 1 to 3 are based on only 30 patients. In table 1, MMS score has been used as a dependent variable, as every traumatic event was considered as an independent variable for the analysis of variance (ANOVA), which is used to compare two groups. Considering that MMS score has not a normal distribution, a cubical transformation has been applied. These statistical analyses have also been performed using the illness duration as a confounding variable, therefore adjusting the MMS scores for all the patients, as if all of them had been analyzed with the same time-span of the disease (hypothesizing a linear evolution with time). This allows to easily identify a possible correlation to the traumatic events. Considering the small number of patients, no more confounding variables have been applied.

Willing to flesh out our initial results, we used another variable, the latency (described earlier in data analysis), to assess the effect of traumatic life events on the worsening of the MMS score, reflecting indirectly the phase of the disease (MCI or AD). The distribution of this variable being normal, no transformation was needed this time. Finally, we performed a correlation analysis, using the Pearson's Coefficient, in order to compare the effect of different variable on the MMS score (see table 3).

A statistical result can be considered as significant when p-value ≤ 0.05.

2. Results

All events confounded, statistics didn't show any significant difference between control cases group and MCI or AD group regarding the number of traumatic event experimented in life regardless of their chronology. Out of 106 memories of traumatic life events, 22 were recalled by control cases, 40 by MCI patients and 44 by AD patients.

Statistics testing the correlation between the experience of a traumatic life event and the MMS score didn't show any significant results (see table 1). None of the traumatic events happened to worsen significantly the MMS of patients (MCI and AD confounded) having experienced it, which denies our hypothesis that traumatic events are triggering the neurodegenerative changes. Surprisingly, results showed quite the contrary, as it seems, according to our data, that there is a small tendency for physical abuse and psychiatric disorder of being protective against a worsening in MMS score, the MMS score of these patients being higher than the one that has not experienced those two traumatic events. However, these results should be interpreted with caution, the numbers of measure being so few (n=2 for physical abuses, n=8 for psychiatric disorders). When non adjusted, the results are even less significant. As seen earlier in statistical analysis, the hypothesis of a linear evolution of the disease with time was made when adjusting the MMS to the illness duration.

When comparing average age and MMS between the groups, we noticed that AD patients are significantly older than control cases ($p \leq 0.05$) and MCI patients have a significantly higher MMS score than AD ones. These two observa-

	NEGATIVE ANSWER		POSITIVE ANSWER		p	
	MCI+AD	MMS	MCI+AD	MMS	Not Adjusted	Adjusted
Spontaneously recalled events (all together)	13 (43%)		17 (57%)			
Spontaneously recalled events after 1984	20 (67%)	22.8 ± 6	10 (33%)	25.0 ± 3.5	0.2693	0.8061
Spontaneously recalled events before 1984	20 (67%)	23.5 ± 5	10 (33%)	23.9 ± 6	0.6638	0.5934
Serious accident	28 (93%)	23.8 ± 4	2 (7%)	20.0 ± 13	0.6973	0.5621
Mental/physical violence	28 (93%)	23.3 ± 5	2 (7%)	26.3 ± 4	0.2325	**0.0336**
Sexual abuse	29 (97%)	23.6 ± 5	1 (3%)	24.0	0.9010	0.6146
Painful mourning	9 (30%)	24.6 ± 3	21 (70%)	23.1 ± 6	0.6152	0.7379
divorce/separation/infidelity	22 (73%)	23.9 ± 4	8 (27%)	22.9 ± 7	0.8684	0.4061
Being witness of a catastrophe	27 (90%)	24.2 ± 4	3 (10%)	18.3 ± 9	0.1067	0.2041
Facing a difficult diagnosis	25 (83%)	23.5 ± 5	5 (17%)	24.5 ± 2	0.9612	0.4617
Difficult treatment / operation / hospitalization	22 (73%)	24.7 ± 3	8 (27%)	19.3 ± 8	0.0651	0.2257
Death Note	30 (100%)	23.6 ± 5	0 (0%)	/	/	/
Missing person	30 (100%)	23.6 ± 5	0 (0%)	/	/	/
Trouble with justice	29 (97%)	23.4 ± 5	1 (3%)	28.0	0.2647	0.2680
Serious financial problem	28 (93%)	23.3 ± 5	2 (7%)	28.5 ± 1	0.0556	0.0559
Dismissal / unemployment / professional threat	24 (80%)	23.5 ± 5	6 (20%)	24.0 ± 4	0.9205	0.3522
Deportation	27 (90%)	23.5 ± 5	3 (10%)	24.5 ± 4	0.7417	0.1547
Psychiatric disorder	22 (73%)	22.7 ± 5	8 (27%)	26.0 ± 3	0.0696	**0.0162**
Other not mentioned	29 (97%)	23.5 ± 5	1 (3%)	24.0	0.9946	0.2049

Table 1: Correlation between the experience of a traumatic life event and the MMS score. MMS scores underwent a cubical transformation in order to obtain a normal distribution and were adjusted, using the illness duration.

	NC	MCI	AD	p
Age [years]	70.0 ± 11.0	72.8 ± 11.9	80.9 ± 9.2	
MMS		28.0	21.0	
Short Latency (pg 1984) [years]		13.8 ± 8.8	21.0 ± 5.3	0.148
Long Latency (pp 1984) [years]		52.2 ± 16.4	56.5 ± 13.4	0.68

Table 2: Age and MMS comparison between groups. Difference of latencies between MCI and AD.

Predictive Variable	Pearson's Coefficient	p
Age	**-0.401692981**	**0.03**
Illness Duration	0.107694305	0.57
Short Latency	0.289545137	0.19
Long Latency	-0.129824222	0.63

Table 3: MMS prediction by some other variables. MMS score underwent a cubical transformation in order to obtain a normal distribution.

tions have their interest in the interpretation of other results. About the statistical tests on latency (see table 2), results show that there is a slight tendency for the events that happened 13 years before the onset of symptoms of MCI patient, and 21 years before the onset of symptoms of AD patient (also known as "short latency"), to be associated with MCI and AD with a difference of approximately 8 years between the two phases of the disease. But after performing a correlation analysis (see table 3) with age and illness duration as comparative variables, we noticed that out of long and short latencies, age and illness duration, only the age can predict significantly a worsening in the MMS score ($p = 0.03$), which suggests that the latency, especially the shorter one, does not or very slightly predict the worsening of MMS score. In other words, the significant result found in table 2 for short latency might be mainly attributed to age, which is the only variable that proved itself to worsen the MMS score of patients.

3. Discussion

On the overall, one of our main limitation in this study was the small number of participants and therefore data to analyze. But many other bias, approximations and malpractices can be discussed in all the phases of this exploratory study, leading to a lack of accuracy in our results. This discussion aims to bring out some of the problems we encountered while talking of the results we obtained and giving some idea for the design of future studies in the field.

First of all, it has to be noticed that our statistical analysis didn't take into account the multiple experience of a same kind of traumatic event (for instance, the experience of three different painful mourning). Only the number of individual recalling events was used in the statistics ($n = 71$) as the total number of traumatic events recalled ($n = 161$) was not. This is a mistake, as it has been described in several studies that chronic distress (that could be due to an accumulation of acute or subacute traumatic life events) may compromise the structure and function of key areas of the hippocampal formation and other limbic regions so that less age-related pathology would be needed to cause dementia [20]. Indeed, the behavioral responses to stressful events involves changes in gene expression in limbic brain regions such as the hippocampus. As mentioned in the introduction, a vicious circle exists between early-life adversity, stress response and future chronic stress, which influences the individual proneness to distress [7], underlying once again the interest to integrate the multiple experience of a same kind of traumatic event in the statistical analysis.This being said, we can talk about the statistics we actually performed.

The first important thing to say is that out of 30 patients, if a correlation between the experience of a traumatic event in life and the occurrence of AD was obvious, it would have been shown in the results. The fact that they did not show anything but slight tendencies can signified two things: either there is no correlation between the experience of a traumatic event in life and the development of AD later on, either there is a very slight tendency only, which we could explore with other studies, with specific, more adapted design and more patients.

About the results presented in table 1, it is clear that there is no correlation between the experience of a traumatic life event and a worsening in MMS scores, quite the contrary, with a very small tendency for physical abuse and psychiatric disorder of being protective against a worsening in MMS score which could be interpreted as a "protective effect" due to repetitive stress. We saw earlier that we must stay cautious with these results, first, because the number of data is very small, second, because these results could be due to the status of the patients themselves, as patients with lower MMS may not remember having suffered from physical abuse, or are just being less conscious of their psychiatric disorder. Our statistician also warned us against the probability of false positive (type I mistakes) which occurs when a high number of statistical tests are performed on very few measures.

Results presented in table 1, more than ruling out a potential impact of traumatic event on the development of AD, questioned us about our way to analyze data, using the MMS as a defined variable. Indeed, the MMS, because it can be affected by so many other causes, the most important one being old age, doesn't help in the diagnosis of AD and therefore in the separation of our two groups of patients. It remains a clinical tool to assess progression and severity in dementia, all kind confound, not only AD, which is the reason why, we must be cautious about the interpretation of our results, as we approximate that AD was related to a lower MMS score. This approximations was based on the results in table 2, showing that AD patients are significantly older than control cases and that MCI have a significantly higher MMS score than AD.

It is in table 2 that we find a slight argument for a potential impact of the experience of a traumatic life event on the development of AD. Indeed, despite a few number of participants our results showed a small significant tendency for events that happened 13 years before the onset of symptoms in MCI patients, and 21 years before the onset of symptoms in AD patients of being associated with the development of the disease. With a difference of 7 years between MCI stage and AD, latency that correspond to the usual latency between this two stages of the disease as shown in Figure 2. Statistics also showed that this study was not powerful enough with 15 patients in each group to obtain significant results. According to this analysis, we should have interviewed 18 patients in each group to improve our results. According to this analysis, we should have interviewed > 18 patients (ideally 25), in each group to improve our results. Further studies would therefore be needed in order to support this tendency. Once again, a criticism can be made about our study design, because of the way we selected our participants. Indeed, in our approxima-

tion, we considered that all the MCI can progresses to AD, which is not the case in reality, where studies showed that the majority of diagnosed MCI does not progress to dementia, even after a 10 years follow-up [21].

Last but not least, it seems, according to our correlation analysis (see table 3), that the small tendency found in table 2 for short latency might be mainly attributed to age, which is, as we have seen before, one of the two main risk factor for the development of AD and of great influence in worsening the MMS score of individuals.

While going through our results, other bias were brought out. We discuss them below, separating them into pre-trial bias and clinical trial bias.

Our qualitative study being an exploratory one in the question of the epigenetic related outcomes in AD after the experience of traumatic life events, it encountered several so-called pre-trial bias during the study design. For instance the definition of risk (having experienced a traumatic event) and outcome (development of AD, which is itself a pathology for which the diagnosis cannot be certain until death and anatomopathological examination), which should be clearly defined, could not be in our work. We noticed for instance, after finishing our interviews phase of the study, that we should have been more accurate in our definition of "traumatic life event". Indeed, such subjective measure can have a high interpersonal variability and arbitrary cutoffs (for instance determine when a mourning become traumatic) makes the distinction between groups difficult (who is "traumatized" who is not?). According to Pannucci and Wilkins [22], this can inflate the observed variance seen with statistical analysis, making a statistically significant result less likely. This observation makes sense in our study, our results showing only slight tendencies ($p \gg 0.05$). A standardized protocol for data collection with training of the interviewer (us) could have minimized the impact of this bias. For instance, we could have used known DSM criteria to evaluate the "degree of traumatism" of our participants, or even more standardized, as it has been already used in several studies, using the measure of neuroticism, which has been proved to be a stable indicator of proneness to psychological distress (because they inclined to mental disorders through epigenetic mechanisms). Indeed, according to studies using data from "the Rush Memory and Aging Project", persons with a high level of distress proneness are 2.7 times more likely to develop AD than those not prone to distress [20]. With a measure of neuroticism we would have improved the accuracy of our results, by giving a higher value to the effect of a traumatic event on a person and not just the presence or not of an event in life.

Another well known pre-trial bias is the selection bias. It happens when exposure and outcome have already occurred at the time individuals are selected for study inclusion, which is the case in our study, where we asked known MCI or AD patients to recall the potential experience of traumatic events in their life.

About clinical trial bias we can say that the one which influenced the most our collection of data was the interviewer bias. It refers to a systematic difference between how information is solicited, recorded or interpreted. In our case, even with the same questionnaire, we could sense a difference between how the two interviewers were performing. The way they decided to determine if a painful mourning was considered as "traumatic" or not, or their different definition of a "traumatic psychiatric disorder", etc. was completely subjective. These differences could also been sense between interviews made by the same interviewer.

4. Conclusion

In the overall, our results didn't show any statistically significant correlation between the experience of a traumatic event in life and a worsening of the MMS later on. Due to the lack of power in statistical analysis, the low internal validity of our exploratory study due to the numerous bias involved, and the ambivalence of some of our results, we cannot conclude anything firmly about the impact of strong psychologically stressful events on the development of Alzheimer disease through epigenetic mechanisms

Nowadays, we tend not to consider AD as a "disease" anymore, but as a more complex entity, including multifactorial conditions, supported by several pathological processes, partially independent, but also interacting one with another, being under the influence of several risk factors, specific, as $\varepsilon 4$ allele coding for APOE for instance, but also very unspecific, such as environmental factors which influence the epigenome in many different ways. The difficulty to link a specific brain change to a specific etiological agent in AD pathology, is leading researchers to think through AD all over again, bringing out new hypothesis such as the growing evidences that the neuropathological changes in brain found in AD correspond to a variable host response which could be a protective answer of the brain to an underlying etiology [23, 24]. Other optimistic studies about epigenetic processes in brain showed that cognitive capacities following neurodegeneration should not be considered as lost but simply impaired by epigenetic modifications of gene transcription, which gives the future of therapeutic drug research for AD a radiant prospect [25, 26].

As for the interest of including the history of traumatic life events in patients complaining of cognitive ongoing symptoms, we would say that using a standardized protocol for data collection such as the degree of neuroticism, which was already used in other studies [20], together with the investigation of the level of distress proneness, would be of more use than a simple listing of potentially traumatic events in order to detect patients at higher risk to develop neurodegenerative diseases such as AD and introduce earlier management, such as specific therapeutic drugs working on the memory reconsolidation process [17].

Appendix

Bonjour, mon nom est Fabio Schipani / Sarah El-Achachi, je suis étudiant(e) en médecine en 5e année, actuellement en plein travail de maîtrise sur la maladie d'Alzheimer. Je travaille en partenariat avec les Professeurs Ghika et Demonet de la Consultation Leenards du CHUV, qui ont dû vous informer de notre appel et s'assurer de votre consentement. Cette interview restera strictement anonyme dans le respect du secret professionnel auquel je suis tenu(e) en tant qu'étudiant(e) en médecine. La durée maximale de cette interview est de 10 minutes. Êtes-vous toujours d'accord d'y participer en répondant à nos questions ?
Les questions que je vais vous poser sont relatives aux événements traumatiques que vous auriez pu ou non expérimenter durant votre vie. En effet, notre recherche se focalise sur ceux-ci, notre hypothèse étant qu'ils pourraient être à l'origine de changements dans le cerveau.

1. Avez-vous le souvenir d'avoir vécu un (ou plusieurs) événement(s) traumatisant(s) durant ces 30 dernières années (depuis 1980 environ) ? Si oui, quand a-t-il (ont-ils) eu lieu ?

2. Je vais à présent vous citer différents événements potentiellement traumatisants. Dites-moi si vous avez vécu l'un ou plusieurs d'entre eux, toujours si ils ont eu lieu durant ces 30 dernières années :

   - Un accident grave ou des blessures graves ? Si oui quand ?
   - Des violences physiques ou mentales ? Si oui quand ?
   - Une agression sexuelle ? Si oui quand ?
   - Le décès d'un proche ou un deuil difficile ? Si oui quand ?
   - Un divorce, une séparation, un abandon ou une infidélité qui vous aie marqué(e) ? Si oui quand ?
   - Avoir été témoin d'un accident grave ou d'une catastrophe ? Si oui quand ?
   - Avoir reçu un diagnostic grave ? Si oui quand ?
   - Avoir vécu un traitement/opération/hospitalisation difficile ? Si oui, quand ?
   - Une menace de mort (directe ou indirecte) ? Si oui, quand ?
   - Une disparition ? Si oui, quand ?
   - Un problème financier grave ou une faillite ? Si oui, quand ?
   - Un problème avec la justice ? Si oui quand ?
   - Un licenciement, un chômage, ou une menace professionnelle ? Si oui, quand ?
   - Un éloignement à l'étranger ? Si oui, quand ?
   - Un problème psychiatrique ? Si oui quand ?
   - Un autre événement que nous n'aurions pas mentionné ? Si oui, quand ?

   (Pour chaque oui :) Souhaiteriez-vous en parler brièvement ?

3. Lors des épreuves, vous aidez-vous d'une croyance religieuse ou autre ?
   Avant de terminer, avez-vous des questions ou souhaitez-vous ajouter quelque chose ?
   Nous sommes arrivés à la fin de notre questionnaire, je vous remercie pour votre collaboration et votre disponibilité. Si vous êtes intéressés par les résultats de notre travail, nous vous communiquerons nos résultats lorsque ceux-ci auront été rédigés. Merci et bonne fin de journée.

Acknowledgments

We acknowledge the professor Ghika, who has provided us guidance through this Master thesis and the "Leenaards Memory Center of the CHUV, Lausanne" for recruiting participants into this study. We also want to acknowledge warmly the professor O. Dériaz, who performed statistics presented in this article.

References

[1] M. Prince and J. Jackson. World Alzheimer Report 2009. *Alzheimer's Disease International*, 2009.

[2] M. Prince, M. Guerchet, and M. Prina. The Global Impact of Dementia 2013–2050. *Alzheimer's Disease International*, 2013.

[3] K. Blennow, M. J. de Leon, and H. Zetterberg. Alzheimer's disease. *The Lancet*, 368(9533):387 – 403, 2006.

[4] Z. S. Khachaturian. Revised criteria for diagnosis of Alzheimer's disease: National Institute on Aging-Alzheimer's Association diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, 7(3):253 – 256, 2011.

[5] J. Mill. Toward an integrated genetic and epigenetic approach to Alzheimer's disease. *Neurobiology of Aging*, 32(7):1188 – 1191, 2011.

[6] K. Gill Alagiakrishnan and A. S. S. Fagarasanu. Genetics and epigenetics of Alzheimer's disease. *Postgraduate Medical Journal*, 88(1043):522 – 529, 2012.

[7] R. E. Puckett and F. D. Lubin. Epigenetic mechanisms in experience-driven memory formation and behavior. *Epigenomics*, 3(5):649 – 664, 2011.

[8] J. Gr´aff, T. B. Franklin, and I. M. Mansuy. Epigenetics and memory. *Biologie Aujourd'hui*, 204(2):131–137, 2010.

[9] D. Mastroeni et al. Epigenetics mechanisms in Alzheimer's disease. *Neurobiology of Aging*, 32(7):1161 – 1180, 2011.

[10] J. Gr´aff, D. Kim, M. M. Dobbin, and L. H. Tsai. Epigenetic regulation of gene expression in physical and pathological brain process. *Physiological Reviews*, 91(2):603–649, 2011.

[11] A. S. Faraz and J. D. Jeremy. Epigenetic mechanisms in memory and synaptic function. *Epigenomics*, 3(2):157 – 181, 2011.

[12] J. Gr´aff and L. H. Tsai. The potential of HDAC inhibitors as cognitive enhancers. *Annual Review of Pharmacology and Toxicology*, 53:311–330, 2013.

[13] K. Koshibu, K. Gräff, and I. M. Mansuy. Nuclear protein phosphatase-1: an epigenetic regulator of fear memory and amygdala long-term potentiation. *Neuroscience*, 173:30–36, 2011.

[14] K. Koshibu et al. Protein phosphatase 1 reglates the histone code for long-term memory. *The Journal of Neuroscience*, 29(41):13079–13089, 2009.

[15] J. Gr´aff et al. Dynamic histone marks in the hippocampus and cortex facilitate memory consolidation. *Nature Communications*, 3:991, 2012.

[16] J. M. H. M. Reul. Making memories of stressful events: a journey along epigenetic, gene transcription, and signaling pathways. *Frontiers in Psychiatry*, 5(5), 2014.

[17] J. Gr´aff et al. Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. *Cell*, 156(1-2):261–276, 2014.

[18] R. A. Sperling et al. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, 7(3):280 – 292, 2011.

[19] G. B Frisoni et al. The clinical use of structural MRI in Alzheimer disease. *Nature Reviews Neurology*, 6:67 – 77, 2010.

[20] R. S. Wilson et al. Chronic Psychological Distress and Risk of Alzheimer's Disease in Old Age. *Neuroepidemiology*, 27(3):143 – 153, 2006.

[21] A. J. Mitchel and M. Shiri-Feski. Temporal trends in the long term risk of progression of mild cognitive impairment: a pooled analysis. *Journal of Neurology, Neurosurgery & Psychiatry*, 79:1386 – 1391, 2008.

[22] C. J. Pannucci and E. G. Wilkins. Identifying and Avoiding Bias in Research. *Plastic and Reconstructive Surgery*, 126(2):619 – 625, 2010.

[23] G. Chételat et al. Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. *NeuroImage: Clinical*, 2:356 – 365, 2013.

[24] R. J. Castellani et al. Reexamining Alzheimer's Disease: Evidence for a Protective Role for Amyloid-β Protein Precursor and Amyloid-β. *Journal of Alzheimer's Disease*, 18(2):447 – 452, 2009.

[25] J. Gr´aff et al. An epigenetic blockade of cognitive functions in the neurodegenerating brain. *Nature*, 483(7388):222–226, 2012.

[26] J. Gr´aff and L. H. Tsai. Histone acetylation: molecular mnemonics on the chromatine. *Nature Reviews. Neuroscience.*, 14(2):97–111, 2013.