UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Master of Medecine - Mémoire

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

Students
Schipani Fabio
El-Achachi Sarah

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

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

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

Lausanne, December 15th, 2014

TABLE OF CONTENTS

1. 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 the expression of thousands of genes, a mechanism called epigenetic[1]. Epigenetic modifications, by modifying genes transcription, help to orchestrate the phenotypical changes linked to development, aging or even diseases and cancer[2]. 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 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. During this study, we tried to identify a possible correlation between the experiencing of a strong psychologically stressful life event, which could have lead to several epigenetic changes, and the occurrence of Mild Cognitive Impairment (MCI) or AD dozens of years later. We also tried to highlight a possible difference in the delay between MCI and AD patients.

2. INTRODUCTION

2.1. EPIDEMIOLOGY

Alzheimer Disease (AD) is a neurodegenerative disease and a major cause of dementia in the world, with a prevalence of 2% at the age of 60-65 years and over 30-35% in people older than 80 years.[3] It is usually diagnosed in people over 65 years old, but in some cases it manifests much earlier.

2.2. PATHO-CLINICAL MANIFESTATIONS

The symptomatology includes inability to acquire new memories, memory loss and cognitive impairment, although there are wide differences between people. In the absence of any known etiology of the disease and any specific biomarker, the diagnostic of AD is defined by clinical, cognitive and functional criteria and can only be definitively confirmed histopathologically with a microscopic examination of brain tissue. Criteria for AD were established in July 1984 by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (commonly referred as NINCDS-ADRDA criteria). Despite being 30 years old, these criteria are still in use, with an estimated sensitivity of 81% and specificity of 70%[4]. A recent reappraisal of these criteria added morphological morphometric and metabolic imaging and CSF specific proteins into the new diagnostic International Working Group (IWG) criteria[5]. It is important to notice that these criteria are based on the assumption that AD is a clinical-pathological entity, meaning that it is supposed to have a perfect correlation between anatomical modifications and clinical manifestations. Today, we know that this isn't the case: a number of cases have shown that before the

symptomatic, clinical phase, there is evidence of an asymptomatic, pre-clinical phase of AD[6]. Based on these assumptions, a natural progression of the disease can be traced:

1) **An asymptomatic pre-clinical phase**, characterized by patho-physiological processes alone. The progress done since 1984 have allowed to identify these modifications as early as 10-20 years before the beginning of the decline of the clinical manifestations (cognitive decline). The main modifications most strongly associated with AD are the followings:

   - Extracellular Senile Plaques, due to amyloïd-beta protein (Aβ) aggregation; detectable by lumbar puncture and manifesting as a low Aβ42 in Cerebro-Spinal Fluid (CSF) and by a positive amyloid imaging at the Proton-Emission Tomography (PET). These are considered to be the earliest detectable modifications.

   - Signs of Neuronal Degeneration, mainly intracellular neurofibrillary tangles due to tau hyperphosphorylation; detectable by lumbar puncture with an increased total tau and phosphorilated tau in CSF. Changes in imaging will follow, such as hypometabolism on the Fluoro-Deoxy-Glucose Positron Emission Tomography (FDG-PET) or less sensitively hypoperfusion on Single-Proton Emission Computed Tomography (SPECT) in the same regions and structural atrophy at specific cortical locations (mesio-temporal, posterior cingulate and temporo-parietal associative cortex) with quantified Voxel Based Morphometry (VBM) at the Magnetic Resonance Imaging (MRI). These are considered late pathological signs of AD.[7]

<table>
<tr><th></th><th>CSF</th><th>Imaging</th><th>Less-specific biomarkers</th></tr>
<tr><td>Amyloïd-β protein</td><td>Decreased Aβ42</td><td>Positive PET-Amyloïd imaging</td><td></td></tr>
<tr><td rowspan="3">Neuronal degeneration</td><td rowspan="3">Increased Tau/Phosphorilated Tau</td><td>Hypometabolism (FDG-PET imaging)</td><td>Inflammatory biomarkers (cytokines)</td></tr>
<tr><td>Hypoperfusion (SPECT imaging)</td><td rowspan="2">Oxidative stress (isoprostanes)</td></tr>
<tr><td>Structural Atrophy (MRI)</td></tr>
</table>

Chart 1: Main AD markers, organized by methodology (upper line) and associated pathological modifications (left column).

2) **A symptomatic, pre-dementia phase**, characteristic of AD, with a clear-cut history of worsening of the cognition but without a significant influence on social and professional activities. We'll refer to this group of patients as Mild-Cognitive Impairment (MCI) due to AD as amnestic MCI were chosen. It usually manifests some years after the pathological modifications, but the precise time-gap is yet unknown.

3) **A dementia phase**, mainly with an amnestic presentation, manifesting as an impairment in learning and recall of recently learned information, and at least another deficit between aphasia, apraxia, agnosia and perturbation of executive functions. The NINCDS-ADRDA and the revised National Institute on Aging/Alzheimer's Association criteria apply to the diagnosis of this phase. It is the most advanced stage of the disease and it always leads to the death of the individual.

2.3. GENETICS/EPIGENETICS

Genetics and epigenetics modifications are key factors in outlining two main groups of patients based on the age of the beginning of the clinical manifestations:

1. Patients with an early-onset of the disease

These patients develop AD at an early age, usually before turning 50 years old, and they represent a small part of the cases (around 5%). Most of these cases are due to one of the 3 autosomal dominant mutations in genes identified as responsible for the development of AD: Amyloid Precursor Protein (APP) on chromosome 21, Presenilin 1 (PSEN1) on chromosome 14 and Presenilin 2 (PSEN2) on chromosome 1[8]. These genes are referred as predictive genes because of their high penetrance of nearly 100%.

2. Patients with a late-onset of the disease

These patients develop AD later in life, usually after turning 65 years old with a prevalence doubling every 5 years, and are the vast majority (nearly 95%). They rarely present a clearly genetic transmission of the disease, whose development is recognized as being mainly due to epigenetic factors. Some genetic factors such as mutations in Apolipoprotein E gene (APOE) are recognized to play a role in late-onset AD, but they are far less predictable than the mutations presented by the early-onset patients (APP and PSEN1-2); for example, it has been estimated that population displaying the APOE mutation for the disease present an increased risk of 4 times of developing AD compared to people with non-mutated APOE. Due to their limited implications in the disease, they are referred as susceptibility genes[9].

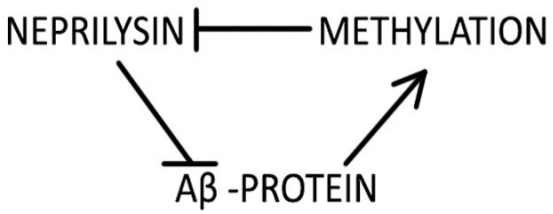


Illustration 1: Vicious cycle of Neprilysin

It appears evident that genetics alone do not fully explain the pathogenesis of the disease (APOE mutation being mainly a simple risk factor). Epigenetic modifications are increasingly recognized as playing a role in the progressive cognitive degeneration of patients affected with late-onset AD; for example, neprilysin, the gene encoding for the main degradation enzyme of Aβ, if silenced via methylation, leads to an accumulation of Aβ protein, which in turn plays a positive feedback in methylation of the sequence (creating a vicious cycle)(Illustration 1)[10].

Whichever external input, being nutrients, infections, smoking or life experiences, can potentially

activate or silence a gene without modifying the genome. Most known epigenetic mechanisms consist in methylation of DNA sequences and modifications (methylation, acetylation or phosphorylation) of histone proteins. These modifications have been shown to appear years before the clinical manifestations and even before the neuropathological changes in dementias, implying that they could be implicated in the initiation phase of AD. It is worth noting that epigenetic modifications are potentially reversible, meaning that understanding the mechanisms behind these changes may lead to the development of an until now nonexistent cure for AD (treatment for AD is, until now, purely symptomatic and it consists mainly in Cholinesterase Inhibitors (Donepezil, Rivastigmine, Galantamine and Tacrine) and NMDA Receptor Antagonists (Memantine)).
Despite not having yet identified which epigenetic modifications are responsible for it, it has been demonstrated that the association between head trauma and APOE mutation leads to a 10 times increased risk of developing AD when compared to patient with APOE mutation and no history of head trauma. In the late 1990s a study showed that electrical stimulation in mice could reduce Brain Derived Neutrophic Factor (BNDF) gene methylation, boosting its activity and consequently promoting new synaptic connections; a similar result has been obtained with administration of a DNA-methyltransferase inhibitor (zebularine), leading to an increase of mRNA levels of BNDF in the hyppocampus. In December 2010, a relation between mice with chronic sleep restriction and an increase of Aβ-plaques formation in their brains has been demonstrated, while another study managed to show how fear stimuli were able to induce epigenetic modifications, going as far as demonstrating that said modifications were more important in aged compared to younger mice[11]. All of these considerations lead to the speculation that stress inducing events may be implicated in the initiation phase of AD. The role of epigenetics has been recently studied in memory processing and suggested to play a role in AD.[12-20] We therefore conducted an explorative study of stressful events in the life of 15 amnesic MCI patients, 15 early AD patients (CDR 0.5-1) and 15 normal controls (NC) and divided them into 2 groups, the ones reporting traumatic events more than 30 years before the onset of symptoms and the ones reporting traumatic events less than 30 years before the onset of symptoms, based on the first preclinical changes recently observed in CSF.

3. METHODOLOGY

3.1. OVERVIEW

The objective of this study consisted in demonstrating a possible implication of old psychologically stressful traumatic situations susceptible to cause epigenetic modifications, important enough to lead to the development of MCI and AD. To this end, we redacted a detailed questionnaire split into two parts: the first one simply consisted in a quick exposure of the argument of the research to the patient and a single open question, directly asking if the patient or care-giver would remember any events that she/he himself would classify as psychologically traumatic occurred in the past; for simplicity, we arbitrarily chose the 30 years before the first symptoms as the line for old and recent traumatic psychological stress. In the second part, we gave a detailed list of psychologically traumatic events and asked the

patient if such occurrences had happened during his life, also asking to specify when. The list of events considered traumatic was as follows:

- **Severe accidents (car, work, …) or serious injuries**
- **Physical violence (aggressions, domestic violence)**
- **Sexual harassment**
- **Death of a close person with a difficult grief**
- **Divorce or separation, abandonment or infidelity**
- **Witness of a grave accident or a catastrophe**
- **Difficult diagnosis (cancer, ...)**
- **Important surgery (cardiac, ...) or difficult hospitalization (radio-/chemotherapy, ...)**
- **Death threats (directly or indirectly)**
- **Person disappearance**
- **Major economic problems, bankrupt**
- **Problems with the law (tribunal, jail, …)**
- **Job loss, unemployment, mobbing, intimidations**
- **Separation from own country**
- **Psychiatric diagnosis/problem (mood disorder, schizophrenia, …)**
- **Another event not mentioned in here**

We ended the questionnaire by asking if the patient has some kind of religious belief and if he wished to add something to the matter; then we'd simply take a leave of our interlocutor thanking him for the disposability and offering him the possibility to receive a copy of the results. All of these information have been collected by the means of telephonic interviews, blind of the diagnosis, that patients and normal controls accepted as a complementary personal history that was incomplete or not explored in their charts.
Our investigation included a total of 45 patients, classified as it follows depending on their characteristics:

1. **Patients affected with AD (15 patients)**
- Diagnosis of late-onset early AD (over 50-years old at the time of the diagnosis)
- Clinical Dementia Rating (CDR) between 0.5 and 1 (meaning they have been just recently diagnosed with these affections)

2. **Patients affected with MCI due to AD (15 patients)**
- Diagnosis of amnestic MCI, pure or with executive functions deficit

3. **Normal controls (NC) not affected with AD nor MCI (15 patients)**
- No diagnosis of MCI or AD
- No other important causes of dementia or other cognitive influencing diseases (schizophrenia...)

The 30 patients affected by AD and MCI have been selected between 2013 and 2014 from the Leenaards Memory Center of the CHUV, Lausanne, Switzerland.
It is worth noting that most of the patients in the AD and MCI groups were still able to fully understand and answer our questions. In occasional cases where the answers were of lesser quality by the patient, the questionnaire was filled with the help of the care-giver or a family member. The patients have been randomized and submitted blindly to us by JG, so that we didn't know the diagnosis until the end of the interviews.

3.2 STATISTICAL ANALYSIS

The results have been split into three sections: Total Events Analysis, MMS Analysis and Latency Analysis.

The results of the Total Events Analysis includes the control cases. Simple calculations were done using EXCEL and OPENOFFICE. A Box Plot has been realized as the conclusion of this section.

The rest of the statistics (MMS Analysis and Latency Analysis) has been mostly performed on data coming from the two groups of persons having respectively a known MMS score (MCI and AD patients) and an established onset of the symptoms, which means that all of the results found in Table of Contents 3 and 4 are based on just 30 patients. These results have been analyzed with STATA.

In every analysis we admitted a statistical result as significant when P was < 0.05 (5%).

4. RESULTS

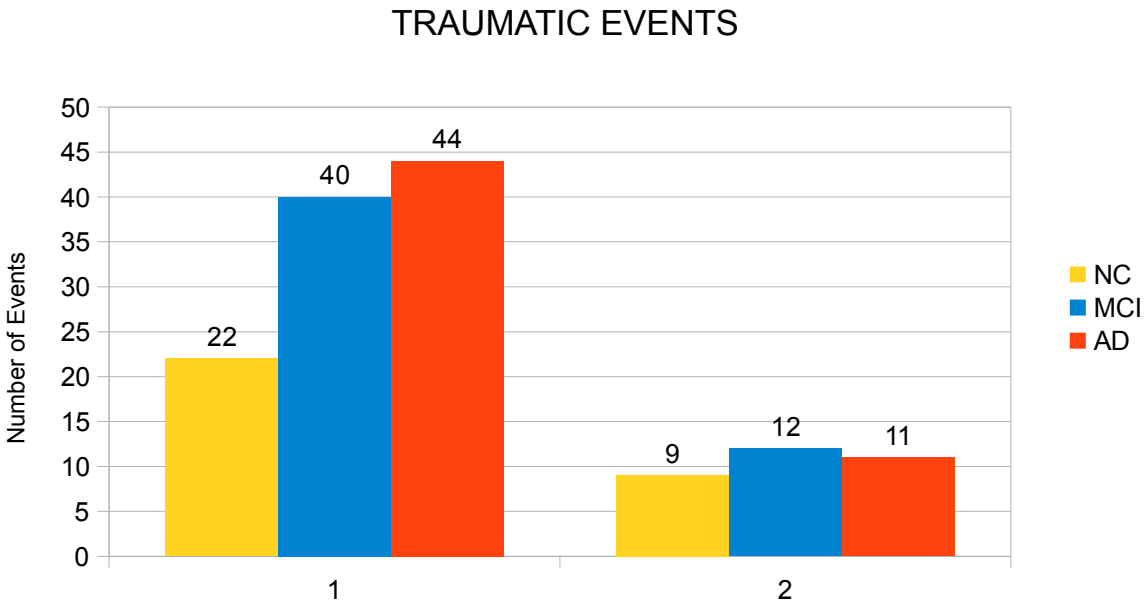
4.1. TOTAL EVENTS ANALYSIS

At the beginning, we tried to simply compare the sum of the traumatic events reported by the patients affected by AD, the sum of the traumatic events reported by the patients affected by MCI and the sum of the traumatic events reported by the NC.

	TOTAL EVENTS [NC/MCI/AD]	% [NC/MCI/AD]
All Events → After 1984 → Before 1984	**106 [22/40/44]** → 58 [15/20/23] → 48 [7/20/21]	
Spontaneously Recalled → After 1984 → Before 1984	**32 [9/12/11]** → 17 [6/6/5] → 15 [3/6/6]	**[40%/30%/25%]** [40%/30%/22%] [43%/30%/28%]
Severe Accident	**3 [1/1/1]**	
Witness a Catastrophe	**5 [2/0/3]**	
Physical Violence	**3 [1/1/1]**	
Sexual Harassment	**2 [1/1/0]**	
Difficult Grief	**41 [8/14/20]**	
Divorce/Separation/Infidelity	**12 [4/4/4]**	
Severe Diagnosis	**7 [2/3/2]**	
Treatment/Operation/Hospitalization	**8 [0/3/5]**	
Major Economic Problem	**2 [0/2/0]**	
Troubles with the Law	**1 [0/1/0]**	
Job Loss/Unemployment/Mobbing	**7 [1/3/3]**	
Separation from own Country	**5 [1/1/2]**	
Psychiatric Disorder	**9 [1/6/2]**	
Death Threats	**0 [0/0/0]**	
Person Disappearance	**0 [0/0/0]**	
Others not mentioned	**1 [0/0/1]**	

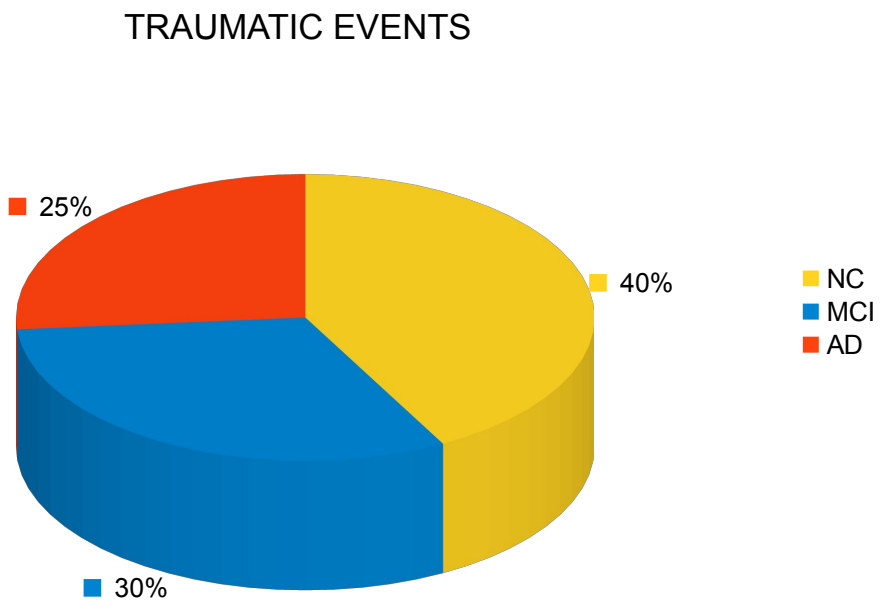
Table of Contents 1: Total events recollected and spontaneously recalled. On the right column, the ratio between the spontaneously recalled and the total events recollected.

The results seem to support the hypothesis that traumatic events may be correlated to the development of AD/MCI, with patients affected by AD reporting more cases (44), closely followed by the MCI (40) and, lastly, the NC (22).



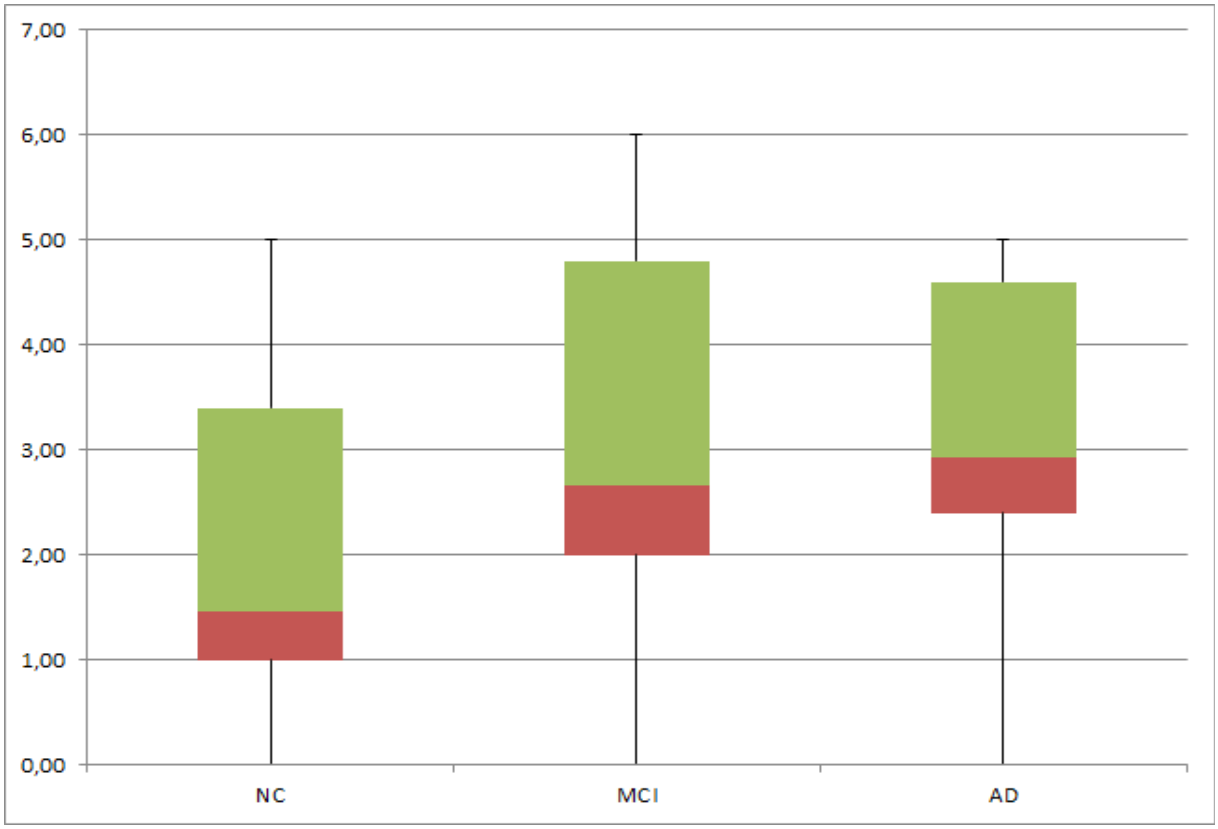
Graph 1: Comparison between status of patients and traumatic events reported (1) or spontaneously recalled (2).

The spontaneously recalled events seem to go more or less in the same direction at first sight, with the NC recalling 9 events, the MCI 12 events and the AD 11 events, but when we consider the fact that NC patients could remember less events (22) compared to the other two groups, it becomes necessary to put them in perspective before analyzing them.



Graph 2: Spontaneously recalled events on total events reported ratio.

To do this, we calculated the ratio of spontaneously recalled events on total events reported. The results are the following: NC: 40% (9/22), MCI: 30% (12/40), AD: 25% (11/44) and they show an inversion of the tendency highlighted with the first results (the more advanced the disease was, the more difficult it was for them to spontaneously remember). This seems to suggest that the patients affected by the disease have more difficulties to spontaneously remember traumatic events, which may in turn suggest that the tendency shown in the reported events could be even more important than what we actually found, considering that the reported events were found interviewing the patients. This, however, is a mere speculation.



Graph 3 (Box Plot): Reported events by status of patients.

By analyzing the results with a box-plot, we noticed that the results tend to be non significant. For a powerful study, at least 25 patients in each group would have been necessary, but we chose to do an exploratory study with 15 patients in each group. We decided, then, to take a different approach: first, we didn't take into consideration anymore the sum of the traumatic events but only the number of patients having experienced at least one of them. Then, we conducted two different analysis. Statistics have been done on the two groups of patients with MMS scores (the AD and MCI patients).

4.2. MMS ANALYSIS

	NC	*MCI*	*AD*
Age [years][1]	70 ± 11	73 ± 12	81 ± 9[3]
"Mini Mental State"[1]		28	21[4]
Spontaneously recalled traumatic events (all together) [yes]	7 (47%)	9 (60%)	8 (53%)
Spontaneously recalled traumatic events after 1984 [yes][2]	4 (27%)	6 (40%)	4 (27%)
Spontaneously recalled traumatic events before 1984 [yes]	3 (20%)	5 (33%)	5 (33%)
Severe accident [yes]	1 (7%)	1 (7%)	1 (7%)
Physical violence [yes]	1 (7%)	1 (7%)	1 (7%)
Sexual harassment [yes]	1 (7%)	1 (7%)	0 (0%)
Difficult grief [yes]	6 (40%)	10 (67%)	11 (74%)
Divorce/Separation/Infidelity [yes]	4 (27%)	4 (27%)	4 (27%)
Witness a catastrophe [yes]	2 (13%)	0 (0%)	3 (20%)
Severe diagnosis [yes]	2 (13%)	3 (20%)	2 (13%)
Difficult treatment/operation/hospitalization [yes]	0 (0%)	3 (20%)	5 (33%)
Death threats [yes]	0 (0%)	0 (0%)	0 (0%)
Person Disappearance [yes]	0 (0%)	0 (0%)	0 (0%)
Troubles with the law [yes]	0 (0%)	1 (7%)	0 (0%)
Major economic problem [yes]	0 (0%)	2 (13%)	0 (0%)
Job loss/Unemployment/Mobbing [yes]	1 (7%)	3 (20%)	3 (20%)
Separation from own country [yes]	1 (7%)	1 (7%)	2 (13%)
Psychiatric disorder [yes]	1 (7%)	6(40%)	2 (13%)
Others not mentioned [yes]	0 (0%)	0 (0%)	1 (7%)
Answered by the patient him/herself [yes]	15 (100%)	15 (100%)	7 (47%)

[1]Average (± Standard Deviation)
[2]Number in the group (over 15) and percentages
[3]AD significantly different from NC ($p<0.05$)
[4]MCI significantly different from AD

Table of Contents 2: Number of patients with spontaneously recalled and with experienced traumatic events for groups of patients.

For the MMS Analysis, MCI and AD groups have been put together, so that we could have statistics on 30 patients (compared to 15 NC). We tried to highlight if the patients who had experienced traumatic events had an MMS score lower than those who didn't. For every traumatic event (for example: medical operations, death threats,...), a possible difference in the MMS score (dependent variable) between the different events (independent variables) has been tested by means of a variance analysis (ANOVA). Considering that the MMS score doesn't have a normal distribution, a cubic transformation has been applied. The duration of the disease has been chosen as a confounding variable, adjusting the MMS scores as if all of the patients had been analyzed with the same time-span of the disease (hypothesizing a linear evolution with the 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.

	NEGATIVE ANSWER			POSITIVE ANSWER		VALIDITY	
	MCI + AD	MMS		MMS	MCI + AD	P not adj.	P adjusted
Spontaneously recalled traumatic events (all together)	13 (43%)				17 (57%)		
Spontaneously recalled traumatic events after 1984	20 (67%)	22.8 ± 6	<	25 ± 3.5	10 (33%)	0.2693	0.8061
Spontaneously recalled traumatic events before 1984	20 (67%)	23.5 ± 5	<	23.9 ± 6	10 (33%)	0.6638	0.5934
Severe accident	28 (93%)	23.9 ± 4	>	20 ± 13	2 (7%)	0.6973	0.5621
Physical violence	28 (93%)	23.3 ± 5	<	26.3 ± 4	2 (7%)	0.2325	0.0336
Sexual harassment	29 (97%)	23.6 ± 5	<	24	1 (3%)	0.9010	0.6146
Difficult grief	9 (30%)	24.6 ± 3	>	23.1 ± 6	21 (70%)	0.6152	0.7379
Divorce/Separation/Infidelity	22 (73%)	23.9 ± 4	>	22.9 ± 7	8 (27%)	0.8684	0.4061
Witness a catastrophe	27 (90%)	24.2 ± 4	>	18.3 ± 9	3 (10%)	0.1067	0.2041
Severe diagnosis	25 (83%)	23.5 ± 5	<	24.5 ± 2	5 (17%)	0.9612	0.4617
Difficult treatment/operation/hospitalization	22 (73%)	24.7 ± 3	>	19.3 ± 8	8 (27%)	0.0651	0.2257
Death threats	30 (100%)	23.6 ± 5		/	0 (0%)	/	/
Person Disappearance	30 (100%)	23.6 ± 5		/	0 (0%)	/	/
Troubles with the law	29 (97%)	23.4 ± 5	<	28	1 (3%)	0.2647	0.2680
Major economic problem	28 (93%)	23.3 ± 5	<	28.5 ± 1	2 (7%)	0.0556	0.0559
Job loss/Unemployment/Mobbing	24 (80%)	23.5 ± 5	<	24 ± 4	6 (20%)	0.9205	0.3522
Separation from own country	27 (90%)	23.46 ± 5	<	24.5 ± 4	3 (10%)	0.7417	0.1547
Psychiatric disorder	22 (73%)	22.7 ± 5	<	26 ± 3	8 (27%)	0.0696	0.0162
Others not mentioned	29 (97%)	23.5 ± 5	<	24	1 (3%)	0.9946	0.2049

Table of Contents 3: Comparison in MMS between MCI and AD patients with and without past traumatic events.

At first glance, it would seem that patients who have experienced some traumatic events (like severe accident or difficult grief) actually have a lower MMS score compared to those that have not. However, the results show, by the means of the descriptive statistics and the ANOVA, that there is no significant difference between the groups (P adjusted to the length of the disease). On the contrary, the following events have shown significant differences:

1. **Physical Violence:** people that have reported it show a higher MMS score. Considering the weak number of cases (n=2), this result has to be considered cautiously.

2. **Psychiatric Disorder:** people that have reported it show a higher MMS score (n=8).

This seems to show that patients who have experienced physical violence or psychiatric disorder in the past could have some sort of "protection" from developing AD/MCI. It is worth noting that for the univariate analysis not adjusted to a confounding variable (P not adj.) there are no differences at all between groups.

4.3. LATENCY ANALYSIS

For the Latency Analysis, we decided to try and analyze the gathered data following another approach by calculating the time between the traumatic events and the onset of the symptomatology, variable that we called "Latency". This, of course, applies only to patients who have experienced the events. The distribution of this variable being normal, no transformation was needed this time. A variance analysis for this variable has been done too, in order to see if the latency was significantly different between the two groups of patients.

	NC	*MCI*	*AD*	*P*
Mean Age	70 ± 11	72.8 ± 11.9	80.9 ± 9.2	
Mean Early Latency (>1984)		13.8 ± 8.8	21.0 ± 5.3	0.148
Mean Late Latency (<1984)		52.2 ± 16.4	56.5 ± 13.4	0.68

Table of Contents 4: Latencies between traumatic events and development of MCI/AD.

It seems there is not a real correlations for the "Late Latency" group (< 1984), having more or less the same time-span between traumatic events/MCI and traumatic events/AD (the P-value is also extremely high). About the "Early Latency" group, however, the results seem to show a shorter latency in patients having developed MCI (13 years) compared to those affected by AD (21 years), reinforcing the hypothesis of the following sequence:

a) Traumatic event -[13 years]→ b) MCI -[8 years]→ c) AD
Traumatic event ----------[21 years]----------→ AD

Despite this, these results too are only a tendency ($P > 0.05$) and we should've analyzed a bigger group of study to obtain more reliable results.

5. DISCUSSION

5.1. OVERVIEW

- Total Events Analysis:
The results obtained from the Total Events Analysis seem to suggest that there is effectively a correlation between traumatic events and the development of MCI/AD, considering that the patients affected by AD and MCI reported a larger number of traumatic events. The fact that the AD (and to a lesser extent the MCI) patients seem to spontaneously remember less traumatic events(compared to the NC) leads to the speculation that the events experienced in their life could actually be more than those actually reported, reinforcing the hypothesis. The limited number of patients, however, limits these conclusions to mere tendencies.

- MMS Analysis:
The results obtained from the MMS Analysis are compatible with the hypothesis that physical violence and psychiatric problems could protect patients from the development of dementia. This seems to point towards the opposite direction we were heading for, namely the development of AD following epigenetic modifications caused by stressful events. A hypothesis to explain this result can be done, that these patients unconsciously developed some kind of "defense" or "resilience" against AD-inducing epigenetic modifications, but it is worth noting, though, that there is a certain number of bias that could have influenced the result (personal reaction towards stressful events, personal, social or educational parameters, Apo E4 phenotype...) that we didn't consider.

- Latency Analysis:
The results obtained from the Latency Analysis show that there may be a correlation between having experienced a traumatic event in the last 30 years and developing MCI/AD, going as far as to identify the possible latencies between the event and the development of the disease. Despite being just tendencies, these results could be used as a basis for further researches in this direction.

5.2 LIMITATIONS OF THE STUDY

We have to acknowledge the limitations of this exploratory study. First of all, the small number of patients, purposely chosen as 15 in each group for reasons of time and availability of consenting patients. The number of patients that would have given the power of statistic potential significant would have been over 25 in each group. Secondly, it is worth noting that it could already be difficult for a person not affected by any particular disease to remember events happened more than 10 years in the past; this is especially true for patients that, despite being at an early stage of the disease, still suffer of a pathology heavily affecting the memory, but stressful events resist more strongly to time in memory. The second part of the questionnaire, with itemized questions asking about specific events, was designed in order to reduce to minimize the impact of this kind of bias. In the specific case of the two seemingly relevant results we have obtained (physical violence and psychiatric problem) it is possible that people with a lower MMS score are less likely to remember traumatic events or are less aware of being affected with a psychiatric disease, and therefore we questioned the caregiver when the quality of answers was suspect of such bias. However, despite knowing the patient very well, the caregiver or family member may simply have a different consideration of what the patient may consider a traumatic event (for example, a grief may be considered traumatic or not, depending on the way it is accepted). In order to further minimize this bias we investigated a little more about the behavior the patient had had after the stressful event (for example, in the context of a grief, a patient may have spent months totally isolated or have changed his relationships with the members of the family). Despite our efforts, there is no possibility to completely exclude this bias. A further limitation is that the patient may simply remember an event as traumatic despite not having affected him at all in the past (he may have simply elaborated it with the time) or having removed the memory of the trauma as some sort of defense despite not having removed the memory of the event. We have not found a way to avoid these last two

bias. However, the patients were interviewed blind of any diagnosis (including normal controls), even though it was not entirely possible to be perfectly blind based on the quality of the answers or their understanding. Another limitation is the time cut-off for old (> 30 years) and recent (< 30 years) stressful events, that we chose in order to potentially cover the theoretical changes in CSF that start about 20 years before onset of symptoms. Data implementations into statistics may have also been reported with minor mistakes. Finally the list of traumatic events we adopted may be incomplete or irrelevant as we do not know the cause of the disease, we must therefore acknowledge that the probability of a type I error (false positive) is also potentially high due to the considerable number of statistical tests.
This study warrants however further work and additional patients in order to answer the main question, since a tendency towards earlier latency between the traumatic event (13 years) for MCI compared to AD (21 years) orients towards a possible link between stressful life events and the development of amnestic MCI and AD compared to normal controls.

6. CONCLUSION

AD is a very common neurodegenerative disease with a purely symptomatic treatment and of mostly unknown etiology. The recent advancements have shown that epigenetic most certainly play a role in the pathogenesis of the disease. We tried with this research to highlight a possible relation between psychologically traumatic events, a known cause of epigenetic modification, and the onset of the disease. Despite not having been able to univocally demonstrate the aforementioned relation, the results we were able to collect seem to suggest that a relation of some kind does exist, but further investigations are needed to clarify its nature.

7. BIBLIOGRAPHY

[1]D. Mastroeni, A. Grover, E. Delvaux, C. Whiteside, P. D. Coleman, J. Rogers. Epigenetics Mechanisms in Alzheimer's disease. Neurobiol Aging 2011 July; 32(7): 1161-80.

[2]A. M. El-Sayed, M. R. Haloossim, S. Galea, K. C. Koenen. Epigenetic modifications associated with suicide and common mood and anxiety disorders: a systematic review of the literature. Biology of Mood & Anxiety Disorders. 2012. Biology of Mood & Anxiety Disorders 2012; 2: 10.

[3]Editorial. Revised criteria for diagnosis of Alzheimer's disease: National Institute on Aging-Alzheimer's Association diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia 7 (2011) 253–256.

[4]Guy M. McKhann, David S. Knopman, Howard Chertkow, Bradley T. Hyman, Clifford R. Jack, Jr., Claudia H. Kawas, William E. Klunk, Walter J. Koroshetz, Jennifer J. Manly, Richard Mayeux, Richard C. Mohs, John C. Morris, Martin N. Rossor, Philip Scheltens, Maria C. Carrillo, Bill Thies, Sandra Weintraub, Creighton H. Phelps. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia 7 (2011) 263–269.

[5] Bruno Dubois, Howard H Feldman, Claudia Jacova, Harald Hampel, José Luis Molinuevo, Kaj Blennow, Steven T DeKosky, Serge Gauthier, Dennis Selkoe, Randall Bateman, Stefano Cappa, Sebastian Crutch, Sebastiaan Engelborghs, Giovanni B Frisoni, Nick C Fox, Douglas Galasko, Marie-Odile Habert, Gregory A Jicha, Agneta Nordberg, Florence Pasquier, Gil Rabinovici, Philippe Robert, Christopher Rowe, Stephen Salloway, Marie Sarazin, Stéphane Epelbaum, Leonardo C de Souza, Bruno Vellas, Pieter J Visser, Lon Schneider, Yaakov Stern, Philip Scheltens, Jeff rey L Cummings. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurology, 2014; 13: 614-629.

[6]Clifford R. Jack Jr., Marilyn S. Albert, David S. Knopman, Guy M. McKhann, Reisa A. Sperling, Maria C. Carrillo, Bill Thies, Creighton H. Phelps. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia 7 (2011); 257–262.

[7]Marilyn S. Albert, Steven T. DeKosky, Dennis Dickson, Bruno Dubois, Howard H. Feldman, Nick C. Fox, Anthony Gamst, David M. Holtzman, William J. Jagust, Ronald C. Petersen, Peter J. Snyder, Maria C. Carrillo, Bill Thies, Creighton H. Phelps. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia 7 (2011); 270–279.

[8]Jonathan Mill. Toward an integrated genetic and epigenetic approach to Alzheimer's disease. Neurobiology of Aging 32 (2011); 1188–1191.

[9]Kannayiram Alagiakrishnan, Sudeep S Gill, Andrei Fagarasanu. Genetics and epigenetics of Alzheimer's disease. Postgrad Med J 2012; 88: 522-529.

[10]V Bollati, D Galimberti, L Pergoli, E Dalla Valle, F Barretta, F Cortini, E Scarpini, PA Bertazzi, and A Baccarelli. DNA methylation in Repetitive Elements and Alzheimer disease. Brain Behav Immun. 2011 August; 25(6): 1078–1083.

[11]Tzong Yuan Wu, Chih-Ping Chen, Tzyy-Rong Jinn. Alzheimer's disease: aging, insomnia and epigenetics. Taiwan J Obstet Gynecol December 2010; 468-472.

[12]Gräff J, Jseph NF, Horn ME. Samiel A, Meng J, Seo J, Rei D, Bero AW, Phan TX, Wagner F, Holson E, Xu J, Sun J, Neve RL, Mach RH. Haggarty SJ. Tsai LH. Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. Cell 2014; 156: 1-2: 261-276.

[13] Gräff J, Tsai LH. Histone acetylation: molecular mnemonics on the chromatine. Nat Rev Neurosci 2013; 14: 2: 97-111.

[14] Gräff J, Tsai LH. The potential of HDAC inhibitors as cognitive enhancers. Annu Rev Pharmaol Toxicol 2013: 53: 311-330.

[15] Gräff J, Woldemichael BT, Berchtold D, Dewarrat G, Mansuy IM. Dynamic histone marks in the hippocampus and cortex facilitate memory consolidation. Nat Commun 2012: 3: 991.

[16] Gräff J, Rei D, Guan JS, Wang WY, Seo J, Henninh KM, Nieland TJ, Fass DM, Kao PF, Kahn M, Su SC, Samiel A, Joseph N, Haggaty SJ, Delalle I, tsai LH. An epigenetic blockade of cognitive functions in the neurodegenerating brain. Nature, 2012: 483: 7388: 222-226

[17] Koshibu K, Gräff J, Bullens M, Heitz FD. Brechtold D, ussig H, Farinelli M, Bollen M, Mansuy IM. Protein phosphatase 1 regulates the histone code for long-term memory. J Neurosci 2009: 29: 41: 13079-13089.

[18] Gräff J, Franklin TB. Mansuy IM. Epigenetics and memory. Biol Aujourd'hui, 2010: 204: 2: 131-137.

[19] Gräff J, Kim D, Dobbin MM, Tsai LH. Epigenetic regulation of gene expression in physical and pathological brain process. Physiol Rev 2011: 91: 2: 603-649.

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

8. ATTACHMENTS

8.1. QUESTIONNAIRE

Here is a copy of the questionnaire used for the telephonic interviews. Because of the patients residing in French-speaking locations, it is done in French language.

Bonjour, mon nom est Fabio Schipani/Sarah El-Achachi, je suis étudiant/e en médecine en 5e année et nous sommes en train de faire un travail de recherche sur la maladie d'Alzheimer avec les Prof. Ghika et Démonet de la Consultation Leenards de la mémoire du CHUV, qui ont dû vous informer de notre appel et vous envoyer un formulaire d'information et de consentement. On sait à présent que les premiers marqueurs de la maladie peuvent se mesurer aussi loin que 15 ans avant le début des premiers symptômes, en analysant, par exemple, le liquide céphalo-rachidien à la recherche de protéines spécifiques. Pour essayer de rechercher un éventuel facteur déclenchant à ce moment ou peu avant, nous recherchons si des événements traumatiques psychologiques graves ou importants (séparation, décès, accident…) survenus entre 15-25 ans avant le début des premiers symptômes pourraient graver un signal dans le matériel génétique (épigénétique en fait) qui pourrait déclencher la maladie. Nous avons réalisé un questionnaire rapide afin de voir si on rencontre de manière significativement plus souvent ce type de traumatismes psychologiques chez les patients atteints d'une maladie d'Alzheimer que chez des gens du même âge non atteints par la maladie ou ayant des troubles débutants de la mémoire. Nous voulons aussi analyser si la chronologie correspond au début des symptômes. Pour cette raison, nous allons interviewer de façon anonyme (nous avons reçu votre téléphone sans le diagnostic) et dans le respect du secret professionnel auquel je suis aussi tenu en tant qu'étudiant. En principe la durée de l'interview est de 10 minutes. Etes-vous toujours d'accord de répondre à nos questions?
Je vais donc vous poser les questions relatives aux événements traumatiques sur lesquels nous sommes en train de focaliser notre recherche.

1. Avez-vous vous même l'impression ou le souvenir d'un (ou plusieurs) traumatisme(s) psychologique(s) grave(s) ou important(s) entre 15 et 25 ans en arrière (depuis 1990 environ)? Si oui lequel vous semble le plus marquant ou important? Quand a-t-il eu lieu?
2. A présent pour voir s'il y a éventuellement d'autres événements qui vous échapperaient en ce moment, je vais vous donner une liste de traumatismes psychologiques possibles et vous me direz si vous les avez vécus ces 30 dernières années:
- Un accident grave (voiture, travail…) ou des blessures graves? Si oui quand?
- Des violences physiques (agression, violence familiale) ou agressions sexuelles ? Si oui quand?
- Un décès d'un proche ou un deuil difficile? Si oui quand?
- Un divorce ou une séparation, un abandon ou une infidélité qui vous a marqué(e) . Si oui quand?
- Etre témoin d'un accident grave ou d'une catastrophe? Si oui quand?
- Une opération ou un diagnostic grave, une hospitalisation (cancer, rayons, chimiothérapie, opération cardiaque, TCC ou autre)? Si oui, quand?

- Une menace de mort (directe ou indirecte). Si oui, quand?
- Une disparition? Si oui, quand?
- Un souci financier grave, une faillite? Si oui, quand?
- Un problème avec la justice (tribunal, emprisonnement…)? Si oui, quand?
- Une perte de travail, chômage, menace professionnelle, mobbing? Si oui, quand?
- Un éloignement à l'étranger? Si oui, quand?
- Un problème psychiatrique (dépression, autre…): Si oui quand?
- Un autre événement que nous n'aurions pas mentionné? Si oui, quand? Souhaiteriez-vous en parler brièvement?

Lors de vos épreuves, vous aidez-vous avec une croyance religieuse ou autre?
Avant de terminer, avez-vous des questions ou souhaitez-vous rajouter quelque chose?
Nous sommes arrivés à la fin de notre questionnaire, je vous remercie pour votre collaboration et votre disponibilité. Si vous êtes intéressés aux résultats de notre travail, nous vous enverrons un résumé lorsqu'il aura été rédigé. Merci et bonne journée/soirée.

8.2. TABLE OF RESULTS [see Attachments 8.2]

8.3. ABBREVIATIONS

AD: Alzheimer Disease
MCI: Mild Cognitive Impairment
NC: Normal Controls
NINCDS-ADRDA: National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
CDR: Clinical Dementia Rating
MMS: Mini Mental State

Aβ: Beta-Amiloïd protein
CSF: Cerebro-Spinal Fluid
PET: Positron-Emission Tomography
MRI: Magnetic Resonance Imaging
SPECT: Single-Proton Emission Computed Tomography

APP: Amyloid Precursor Protein
PSEN1: Presenilin 1
PSEN2: Presenilin 2
APOE: Apolipoprotein E
BNDF: Brain Derived Neutrophic Factor