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PILOT STUDY

Oxytocin receptor polymorphisms and attachment style in patients with cognitive impairment and affective symptoms

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BACKGROUND: Behavioural and psychological symptoms (BPS) occur frequently in patients with dementia. Many reviews have highlighted that BPS can be influenced by the subject's personal attachment style (AS). Then, recent studies have found that oxytocin (OT) appears as a marker of emotional intensity and attachment. Our approach is based on 2 hypotheses: a) OT should decrease in MCI (mild cognitive impairment) patients, b) there are correlations between affective BPS, insecure AS and OT receptor polymorphisms.

METHODS: The present study assessed both OT blood levels and OT receptor polymorphisms (OTrx) in MCI patients. 9 MCI subjects and 8 controls are included. AS, BPS and depression were assessed using standardized scales. OTrx were determined using PCR.

RESULTS: All MCI patients showed an inhibited attachment style profile in one's relationship to a parent. Insecure AS and OTrx were found in the two MCI patients presenting with the most severe affective BPS.

CONCLUSION: These results are in favour of our hypotheses (OTrx rs53576 and insecure AS are found in patients with severe BPS). Then, we found an inhibited attachment profile in patients with MCI, but it must be confirmed in the future, with a large sample of subjects. On one hand, our experiment can be used to make the study possible for a larger number of subjects. On the other hand, the need of a longitudinal study has to be done.

Key Words: *Cognitive Impairment; dementia; oxytocin receptor polymorphisms; Attachment; life course; genetic variations; behavioural phenotypes.*

Abbreviations: *BPS: Behavioural and psychological symptoms; OT: Oxytocin; OTrx: Oxytocin receptor polymorphisms; CDR: Clinical Dementia Rating; AS: Attachment style (profile); HAD: Hospital Anxiety and Depression scale; RAID: Rating Anxiety in Dementia; NPI: Neuro Psychiatric Inventory (assessment); MCI: Mild Cognitive Impairment; AMMI: Attachment Multiple Model Interview (AMMI)*

Behavioural and psychological symptoms (BPS) of dementia occur in most patients with dementia [1,2]. BPS, due to a complex and multifactorial etiopathogenesis, often occur in both mild cognitive impairment and dementia. Indeed, risk factors are multiple and include biological, psychological and environmental variables. Frequently, their combination explains the occurrence of BPS in an individual patient. Studying affective BPS, we have argued premorbid personality and attachment style can help explain BPS during dementia [1-3]. Biological factors (e.g. cerebral lesions and altered neurotransmitters, comorbidities), may interact with psychological aspects (e.g. attachment, personality, social well-being). To our knowledge [4], insecure attachment (acquired during infancy) is sometimes linked with anxious disorder as well as depression in adulthood. Some studies [1-5] have shown that insecure attachment (through social behaviour) [5] as well as personality traits (e.g. behavioural inhibition [4], neuroticism) [5] may explain BPS in patients with cognitive impairment. Moreover, neurohormonal and neurotransmitters or their changes are involved in the occurrence of BPS [1,2]. Oxytocin (OT) is one of them [1,6-10].

These last several years, research on attachment suggests that OT may be a determinant of attachment characteristics [6-9]. Indeed, OT could play a key role in modulating social attachment and affiliate behavior [8]. Cerebral regions, which are involved in social behaviour related to OT, are raphe nuclei, right amygdala, hippocampus and orbitofrontal cortex. Mottolese [6] has found these regions, studying OT administration in healthy subjects using the Positon Emission Tomography thanks to a radiotracer specific for 5-HT. Then, intranasal administration of OT in humans has both favorable effects on social anxiety symptomatology [6-9] and cognitive impairments (for example, in frontotemporal dementia) [10]. These results strengthen the role of OT in social cognition and social behavior. OT significantly attenuates the amygdala's reactivity [7,11,12] thereby reducing the orienting response to social cues (angry or neutral faces) [9,13]. Finally, Stoop and Viviani et al. summarize that "OT selectively gates fear responses through distinct outputs from the central amygdala projections emerged from separate neuronal populations respectively to hypothalamic and brain stem nuclei"[12]. OT leads to an inhibition of fear and improves trust. Then, Oxytocin receptor with single nucleotid polymorphisms (SNP) rs53576 and rs2254298 (OTrx) are sometimes involved in affective disorders, such as depression as well as social anxiety or separation anxiety [14,15]. Moreover, correlations have been found between higher amygdala volume and rs53576 presence, underlying stress reactivity and neuroticism. In the same way, Sippel et al. [16] indicate that the interactions between polymorphisms rs53576 and insecure attachment may contribute to vulnerability to post-traumatic stress disorder. According to Schiele et al. [17], OT rs2254298 has a role in complicated grief, in separation anxiety, (especially if traumatic life event has occurred in children) with behavioural inhibition, since childhood [4].

Therefore, from what precedes, I have hypothesized that: a) OT would decrease in MCI subjects presenting with insecure or avoidant attachment style; b) OTrx polymorphisms rs 53576 and rs2254298 should be involved in MCI with affective BPS. Indeed, OT concentration [18] and OTrx polymorphisms [16,17] should be two neurobiological markers of BPS risk in MCI (Figure 1).

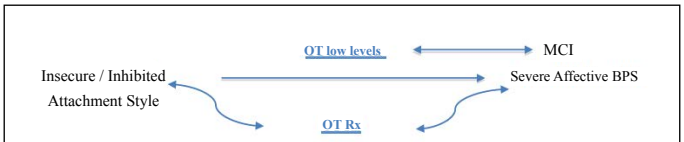

Figure 1) *Hypotheses.*

MATERIALS AND METHODS

Subject

This pilot study grafted upon a more comprehensive study conducted at Lausanne (Lausanne University Hospital) was approved by the ethics

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committee of Canton of Vaud. Nine patients with mild cognitive impairment (MCI) and eight healthy volunteers over 60 years participated in the study. For each subject and his relative, informed and written consent was obtained. We have explained to each subject and proxy the goals of this project. Patient recruitment was performed at the at outpatient consultation of the Old-Age Psychiatry and the Leenards Memory Clinic of the Lausanne University Hospital. The recruitment of a cognitive healthy elderly control group was done by mouth of word and local advertisements in the above centers. All interviews were conducted at Outpatient Consultation Service of Old Age Psychiatry.

Inclusion criteria for MCI patients

- Patients >60 years
- Diagnosis of MCI; CDR=0, 5
- Type of MCI is amnestic MCI–single or multi domain
- Possible AD, with or without subcortical vascular impairment
- Informed consent and patient has a proxy
- Patient accepts drawing blood for oxytocin analyses

Exclusion criteria for MCI patients

- Presence of a major psychiatric except for dysthymia or mild depression
- Presence of a neurological CNS disorder (epilepsy, stroke, tumor)
- Alcohol or drug abuse or dependency or presence of a severe physical illness.

Inclusion criteria for control

- Patients >60 years
- No cognitive disorder diagnosis; CDR=0,
- Informed consent and patient has a proxy
- Patient accepts drawing blood and oxytocin analyses

Exclusion criteria for control

Presence of a major psychiatric or neurological CNS disorder (epilepsy, stroke, tumor)

Presence of MCI or dementia

Alcohol or drug abuse or dependency or presence of a severe physical illness.

PROCEDURE

Psychopathology and medical interview

We used the Mini International Neuropsychiatric Interview – French version 5.0.0 – to exclude patients or controls with major psychiatric disorders. Then, I made an interview on medical comorbidities using the Charlson Comorbidity Index. Finally, I checked for the absence of stroke, tumor or epilepsy in the medical records. The treatment was listed. Traumatic life events (since childhood into nowadays) were checked.

Cognitive and functional assessment (Neuropsychological assessment battery) [1]

To allow for early cognitive impairment that is an inclusion criteria for MCI patients, both subjective and objective cognitive impairment must be assessed as well as daily living functioning. The ADL (activities of daily living by Katz, about physical performance and autonomy) and IADL (instrumental activities of daily living) have been administered to each relative to investigate functional performance, which should be intact to be compatible with an MCI diagnosis (IADL=8/8; ADL=6/6). The CDR (Clinical Dementia Rating), a 5-point standardized scale, which characterizes six domains of cognitive and functional performance reflecting the level of the neurocognitive disorder from 0.5 (MCI) to 3 was completed. The CDR explores the following domains: memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. The subjects have undergone a thorough neuropsychological assessment. We used the following tests to assess general cognition: MoCA, (the Montreal Cognitive Assessment is a widely used screening assessment for detecting cognitive impairment); the Grober Buschke test (spontaneous and cued recall – 16 items) to evaluate episodic memory. The Stroop task was used to evaluate inhibition, the Letter and category verbal fluency to assess initiation, the trail making test part B to evaluate another executive function. The trail making test part A was used to evaluate processing speed and the brief standardized validated French screening scale was used to evaluate praxic abilities.

Assessment of affective symptoms and BPS [1,2]

The Hamilton anxiety and depression scale (self-evaluation). According to the literature, a cut-off for caseness of at least 10 was used (scale range 0–21).

The NPI questionnaire explores 12 dimensions of BPS, i.e. anxiety, depression, apathy, irritability, elations, disinhibition (affective BPS), with or without agitation/agressiveness, wandering, sleep disturbance, eating disorders or psychotic symptoms such as delusions, hallucinations. Severity (score from 1 to 3) and consequences (impact on relative from 0 to 5) are noted interviewing the relative.

The RAID (Rating Anxiety in dementia) and Cornell scale were employed to assess respectively anxiety and depressive symptoms in dementia or MCI.

Assessment of attachment style: The Attachment Multiple Model Interview (AMMI) [19]

In our study, all attachment interviews were recorded. The AMMI assesses internal working models of specific relationships (e.g. mother, father, and romantic partner). AMMI dimensions for each subject in a specific relationship are the following:

Secure attachment represents good affective well-being and a high interindividual trust level.

Inhibited attachment (Bowlby,1980) represents a turning away from attachment (as a behavioural way to show no attachment or affective need in a relationship); the subject does not talk about their needs. Moderate scores are given when avoidance is only behavioural (« I pretended I didn't care »), while high scores are given when avoidance is also noted ; so, the subject does not admit and think about attachment-related issues or feelings (« I didn't care anyway »).

Hyperactivation of the attachment has been defined as an « increased contact seeking with attention most exclusively focused on the attachment figure » (Main 1990, Fleming 1993) [19].

The AMMI is a validated instrument. Its structure was developed based on the Adult Attachment Interview (AAI). The AAI and AMMI were used in clinical studies, in order to evaluate accurately secondary strategies about inhibited (e.g. deactivated) and activated attachment, for a specific relationship. These strategies (inhibition vs activation) are found in all relationships in anyone as a property of the person rather than specific relationships. Indeed, previous prospective studies 19 have shown that the AMMI, administered since infancy, has good reliability into adulthood. For each dimension (inhibition, activation and security), score ranges from 0 to 8.

Personality assessment- Big Five Inventory

The BFI is a well-known and validated scale to measure 5 personality characteristics including extraversion, agreeableness, conscientiousness, neuroticism and openness to experience. Behavioural inhibition, a temperamental genetic characteristic [4], is also evaluated by the BFI and AMMI.

OT neuropeptide and OTrx (OT receptor polymorphism) by PCR

A blood test was realized at 9 am (for circadian rhythm). Blood samples were stored in a cooler before being quickly transported to the neuroscience laboratory in order to keep all samples in a -80°-freezer.

Two single nucleotid polymorphisms (SNP) rs 53576 then rs 2254298) were studied by Polymerase Chain Reaction with a thermal cycler (see https://www.thermofisher.com/ch/en/home.html online about these two polymorphisms,). PCR is a technic used in molecular biology to amplify DNA polymorphism, then to identify a SNP by the following steps in particular thermic conditions (for 20 to 40 cycles with temperature >60°C) for each blood sample (volume 200. 10-6 L). The individual steps (by an automated thermal cycler, see: https://www.thermofisher.com/ch/en/home/life-science/pcr/thermal-cyclers-realtime-instruments/thermal-cyclers/automated-thermal-cycler-atc.html) are as follows:

DNA denaturation is a process in which DNA and proteins lose their quaternary structure; denaturation of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.

Annealing step the reaction temperature is lowered to 50°C for 40 seconds, allowing annealing of the SNP primers containing the DNA target region.

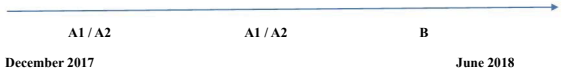
Amplification by DNA polymerase on coloured primers to mark OTrx polymorphisms

Results are compared to a control sample and any DNA contamination is checked.

Measuring OT Neuropeptide by Spectrophotometry

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance like OT. A spectrophotometer is measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected.

Timing of the assessment is described here:



A1: informed and written consent, medical interview, cognitive assessment, BPS assessment. A2: blood test/attachment interview and the relatives' evaluations filled in. B: OTrx polymorphisms by PCR.

STATISTICAL ANALYSES

A Mann-Whitney non-parametric test is appropriate to compare attachment style scores in each dimension (inhibition, security, activation) between MCI and control groups, between MCI with OTrx and MCI without OTrx polymorphisms. This test was also used to compare affective BPS score (NPI, Cornell, and RAID) in the following groups: MCI with each OTrx polymorphism vs MCI without OTrx polymorphism. Indeed, first there are no matches for age and gender between MCI and control groups. Then, number of subject in each group is not enough to do parametric tests (9 MCI vs 8 controls).

RESULT

MCI patient's vs controls

9 men and 8 women are included. First, there are no matches for age or gender between MCI and Controls, in each group (men and in women). However, each MCI group (men, women) is respectively comparable to controls. Table 1 shows the studied population characteristics (MCI vs Controls, in Men and Women) about age, educational level (from 1 school <12 years; to 3, high school or university degrees), living alone or with relatives. Men have higher educational level than women do. Cardiovascular risk factors equally found in MCI and Controls. One control man had presented a coronary disease. MCI men and women are a little bit older than controls (average: 76, 8 and 75, 6 years in MCI; 70,5 and 71,3 years in Controls). Finally, 3 MCI patients were alone without relatives, whereas all controls lived with their family. We found stressful life events in MCI (n=3) and controls (n=2), for the past five years. Two MCI men and one control woman have had their spouse who has died two years ago. A control man has had a cardiac infarction; a MCI woman had moved that year, and her grandson has died by accident a few years ago. A MCI men had his wife has died that is clearly leaded to BPS.

TABLE 1

MCI patient's vs. Controls

	Men		Women	
	MCI (n=6)	Control (n=4)	MCI (n=3)	Control (n=4)
Age (average)	76,8(67-84)	70,5(63-82)	75,3(60-86)	71,3(69-77)
Educational Level*	2,7(2-3)	2,5(2-3)	2(1-3)	2(1-3)
Single	n=4	n=1	n=2	n=3
No presence of relatives	n=2	n=0	n=1	n=0
Cardiovascular risk factor>2	n=3	n=2	n=1	n=1
Coronary disease	n=0	n=1	n=0	n=0
Stressful life events	n=2	n=1	n=1	n=1

*Socio-educational levels: 1=obligatory school 2=middle school 3=high school

Cognitive characteristics found in MCI patients

Table 2 shows the results of the cognitive assessment. All MCI subjects have an altered episodic memory (observed during Grober Buschke test and also MoCA assessment), associated or not with others impaired cognitive functions (inhibition n=3; verbal fluency n=4; orientation n=3 in MCI). All control subjects performed well on each test, during cognitive assessments. The MoCA test distinguishes MCI subjects from the others, even if scores are not always found pathological (sometimes scores are over 26 in MCI). Then, all subjects are autonomous and independent in everyday life (BADL=6/6; IADL=6/8 at least). These results confirm there is no major neurocognitive disorder (no dementia) and CDR confirms MCI diagnostic.

TABLE 2

Cognitive assessments in MCI and controls

	MCI (N=6)	Control (N=4)	MCI (N=3)	Control (N=4)
Impaired Grober Buschke test (episodic memory)*	n=6	n=0	n=3	n=0
MoCA score	25,3(21-28)	29,25(29-30)	24,7(24-26)	27(26-28)
Impaired verbal fluency test*	n=4	n=0	n=1	n=0
Impaired gestual praxies test*	n=1	n=0	n=1	n=0
Impaired TMT B test* (mental flexibility)	n=2	n=0	n=2	n=0
Impaired TMT A test* (processing speed)	n=2	n=0	n=2	n=0
Impaired Stroop test (inhibition)*	n=2	n=0	n=1	n=0
Impaired orientation*	n=2	n=0	n=1	n=0
IADL score	7,7(6-8)	7,75(7-8)	8	8
BADL score	6	6	6	6
CDR (= 0.5)*	n=6	n=0	n=3	n=0

*n=number of subjects with impaired cognitive test

Affective BPS

Figure 2 shows the clinical data on affective BPS found in 5 MCI patients. For these 5 patients, we find depressive (Cornell, HAD) as well as anxious symptoms (HAD, RAID). However, only two patients present severe distress using the NPI; these two subjects have the most severe affective symptoms (Subject MCI 1 and Subject MCI woman) in the MCI group, whatever scales is used to assess BPS. As severity of BPS increases, distress and suffering increase on the NPI, for these two subjects who have the rs53576 AG carriers. As no surprised, there are correlations on one hand, between Cornell and HAD depression, and on the other hand, between RAID and HAD anxiety. However, the NPI does not systematically highlight these symptoms, but their repercussions (interviewing the relatives). Finally, there is no correlation between BPS and cognitive impairment level. Neither delusion nor hallucination has been found in MCI patients. We found insomnia, depressive symptoms, anxiety and autonomic activity (sub-scale in RAID) in the 5 subjects. In the control groups, there are neither pathological anxiety nor significant depressive symptom (Cornell <7 or RAID <10).

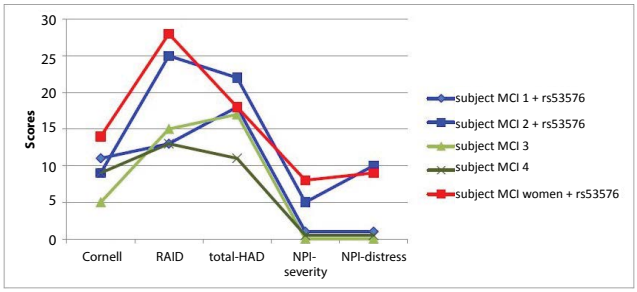


Figure 2) Affective BPS (significant scores in each scale) in MCI (n=5).

Oxytocin receptor polymorphisms and affective behavioural and psychological symptoms

The results of OTrx genotyping are presented here (Table 3). First, rs53576 AG carriers are more frequent in MCI men than in controls, whereas no MCI

men have the rs2254298 polymorphism. Frequencies of rs53576 AG+ are higher in MCI patients (66%), than those found in the general population (39%), (cf. thermofisher online in Methods chapter). Then, rs2254298 (AG or AA) is often found in controls (3 men out of 4, and 1 woman out of 4), that is particularly frequent in that control group men (75%), but not in the MCI group here (n=1 MCI; frequency=11, 1%).

Most MCI patients (n=6 out of 9 MCI subjects; n=4 out of 6 MCI men) and patients with BPS (n=3 out of 5) often have the rs 53576 OT rx polymorphism in that MCI population. Two subjects who have severe affective BPS with moderate scores on NPI dimensions (severity and distress) have the rs 53576 OT rx. However, no subject with BPS have the rs 2254298 carrier.

TABLE 3

Frequency of rs 53576 and rs 2254298 oxytocin receptor polymorphisms

Population	rs 53576 AG +	rs 53576 AA +	rs 2254298 AG+	rs 2254298 AA+
MCI Men BPS+ n=4	n=2	-	-	-
MCI Men (no BPS) n=2	n=2	-	-	-
Control Men n=4	n=2	-	n=2	n=1
MCI Women BPS + n=1	n=1	-	-	-
MCI Women (no BPS) n=2	n=1	-	n=1	-
Control Women N=4	n=1	n=1	n=1	-
General population	39%		21%	

Attachment style

Inhibited attachment (with a parent) has been found in all MCI patients (men and women) (Figure 3). Only one MCI woman has not an inhibited attachment with her father. A statistically significant result ($p<0.05$) was obtained, studying inhibited attachment with the mother, in MCI men, compared to control group (Mann-Whitney test studying median scores). We could not use the Mann-Whitney test in women because of the low number of subject (n=3 MCI women). Then, we don't observe a statistical difference for the intensity of inhibited attachment style with the spouse or partner, in MCI (men and women) compared to controls.

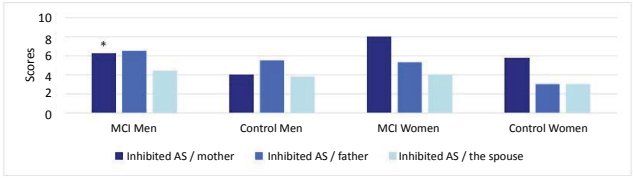


Figure 3) Inhibited Attachment Style Scores (average) in MCI patients and Controls (AMMI score).

(*) statistically significant result $p<0.05$ compared MCI men (n=6) to control men (n=4), about inhibited AS with the mother (Mann-Whitney test studying medians, non-parametric test).

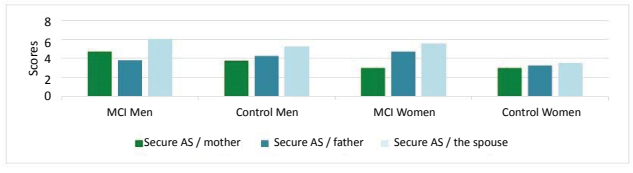


Figure 4) *Secure Attachment Style scores (average) in MCI patients and Controls (AMMI score).*

As shown in Figure 4, there are no differences between each MCI group and the respective control group (men / women), regarding secure attachment. Secure attachment scores are low (from 3 to 4,7), with each parent, but not with the partner, in MCI patients. Indeed, MCI men and women have high secure attachment scores (respectively 6 and 5,5 out of 8) in the relationship with the partner, rather than in the control men group (5,25 of average) or in the control women group (3,25 of average).

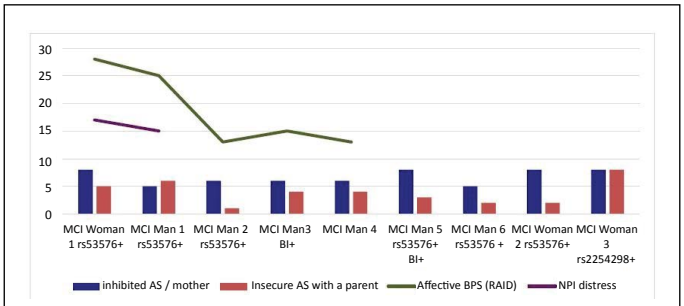


Figure 5) Secure Attachment Style (Scores) in MCI patients and Controls

Figure 5 shows the 5 MCI subjects who have significant affective and anxious symptoms (green color). We found insecure attachment style with a parent in two subjects who have the most severe BPS (MCI Woman 1 and Man 1) These two patients have severe distress and suffering (on the NPI, in violet colour); they have the rs53576 OTrx (AG+ carrier). Otherwise, four MCI subjects have no BPS and are not insecurely attachment with a parent, nor do they have the rs53576 polymorphism. Two MCI men (number 3 and 5) presented behavioural inhibition BI+ (as a well-known risk factor to anxiety). Subject MCI 3 has BI+ and moderate insecure attachment without rs53576 OTrx; he has moderate affective BPS without distress on the NPI. Finally, MCI subjects who experienced the most severe affective BPS (n=2) have insecure attachment and rs53576 OTrx with higher neuroticism. Two others MCI subjects (with BPS) have insecure attachment (n=2) with behavioural inhibition BI (n=1). Only one MCI subject (with BPS) has not insecure attachment, but he presented rs 53576 with a recent life event (his wife died two years ago).

The Big five inventory

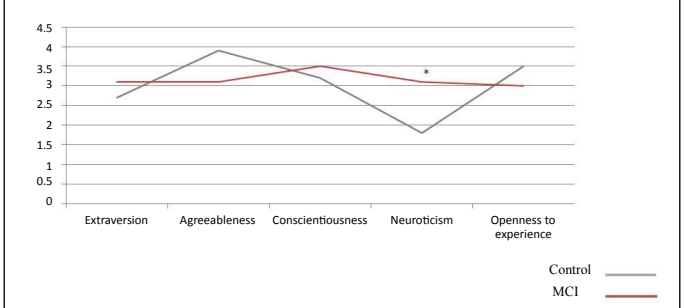


Figure 6) *Big Five Inventory. MCI compared to controls on the Big Five dimensions.*

(*) statistically significant result, $p<0.05$ (Mann Whitney test studying medians, non-parametric test) in MCI group (n=9) compared to control group (n=8).

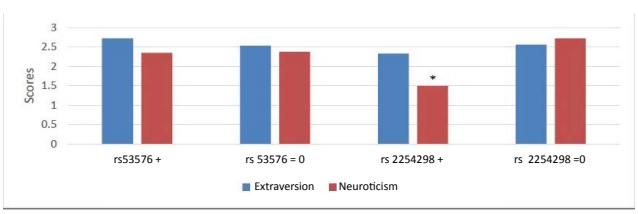


Figure 7) *Presence of OT receptor polymorphisms on neuroticism and extraversion in all subjects.*

(*) statistically significant result $p<0.05$ (Mann Whitney test).

Personality assessment using the Big Five inventory (relative French version) showed higher neuroticism (statistically significant result) in the MCI

group compared to controls (cf. Figure 6). In addition, two subjects have behavioural inhibited temperament (they were afraid of novelty situations in childhood), assessed with the AMMI. They have low openness to experience, low activity and extraversion, but higher neuroticism, and all belong to the MCI group.

Figure 7 shows no correlation between the presence or absence of 53576 and extraversion or neuroticism levels. However, regarding rs 2254298 that is not present in MCI men, we found an opposite link between the neuroticism and the rs2254298 presence (significant difference) here.

OT blood level dosage

For the time being, we have been unable to realize that test, because of a technical problem; OT is bound to many plasma proteins (macromolecules). Anti-oxytocin antibody is needed to fix OT, before spectrophotometry. This technique is still being tested (and therefore not yet approved). Although exogenous OT was well measured in veinous plasma, endogenous OT cannot be detected in plasma. Thus, we are still working solve this technical difficulty.

DISCUSSION

Summary of the clinical and neurobiological results

- Here, the episodic memory is altered for all MCI subjects.
- OTrx 53576 is often present in MCI, with or without affective symptoms (BPS).
- All MCI patients show an inhibited attachment style profile in one's relationship to a parent.
- Higher Inhibited attachment (with a parent) is found with statistical significance ($p<0.05$) in MCI men.
- Insecure Attachment and OTrx rs53576 were found in the 3 MCI patients having the most affective BPS.
- OTrx 2254298 is not involved in MCI or in affective BPS, but linked with a low neuroticism.
- There is no link between OTrx and attachment style.
- Neuroticism is higher in MCI than in Controls ($p<0.05$).

Firstly, the following hypothesis has been confirmed: there is a link between severe BPS and insecure attachment in subjects who have rs53576 (AG+ carrier). Moreover, there are some interesting observations: MCI patients are older than controls, and they are often alone, that confirm our clinical observations in practice. We found stressful life events in MCI (n=3) and controls (n=2), for the past five years. Here, stressful life events are not systematic found in patients with BPS, as it is consistent with the literature [1-3,20]. Regarding the occurrence of BPS, we found no psychotic symptoms, which is keeping with the objective to recruit only subjects with anxiety or depressive symptoms. When there are acute psychotic symptoms related to MCI or dementia, patients often refuse clinical studies because of the disease.

BPS occurs frequently throughout the course of MCI and dementia. Here we can say there is an expected number of patients who have BPS (n=5 out of 9). Two of them have important NPI scores. Affective symptoms such as anxiety, depression, and insomnia are found: these symptoms may worsen the course of dementia [20]. We have found insecure attachment (n=4 out of 5 MCI with BPS), which is a known risk factor for later anxiety and depression in adulthood [4,20,21,23] Insecure attachment is a likely risk factor to affective BPS in MCI as well [1-3,21,23]. So, the combination of risk factors (insecure attachment and OTrs 53576, or BI as well) may help explain the occurrence of affective BPS, in an individual patient [2,3].

rs53576 polymorphism was found in MCI, rather than in the general population. The rs53576 was linked with separation anxiety in adulthood, post-traumatic stress disorder and depression, mediated by stress reactivity and neuroticism [15-17,22]. Our results are in the line with these observations, and in favour of our hypothesis that rs53576 OTrx may be a risk factor to affective disorder. The rs53576 genotype "may present a greater biological sensitivity as well as stress reactivity" according to Chang et al. [22]. This polymorphism would prevent the RNA polymerase from recognizing the RNA chain. Accordingly, a single SNP can predict most of the variance in OT receptor expression in specific brain regions [24]. Indeed, rs 53576 (AG+ or AA+ carriers) is less expressed in the cerebral regions which are involved in social behaviour related to OT. Thus, it leads to decrease OT activity in specific brain structures (accumbens nuclei and limbic structures involved in social attachment), leading to anxiety through the high amygdala's reactivity [6,23,25]. Although we have found no link between rs53576 (AG+ carrier) and personality traits (neuroticism, extraversion), like other authors who didn't find any correlation between the rs53576 (AG+) and the Big Five inventory [25,26] the rs53576 OTrx (particularly AA+ carrier) could be linked to the sociability/extraversion (underlying by dopaminergic system interaction with OT on accumbens nuclei) [26].

Regarding the rs2254298 carrier, this OTrx is not present in MCI men, but only one MCI woman. Indeed, in our population, no subject had an anxiety disorder (panic disorder or social phobia). This polymorphism rs2254298 might be involved in affective disorder, or in separation anxiety with behavioural inhibition, a temperamental characteristic as a genetic risk factor to anxiety disorders, acting through high amygdala's reactivity. Our two subjects with BI have no rs2254298 OTrx. In addition, this rs 2254298 carrier is linked with affective disorders if stressful life events have occurred during childhood. To our surprise, we found a significant link between this rs2254298 OTrx and a low neuroticism in controls compared to subjects without rs2254298. However, we can't conclude because of a recruitment bias (there is no affective disorder in MCI patient here). If this were confirmed in other studies, it would mean rs2254298 is a confounding factor with BI. Otherwise, the stress life during infancy mediated by this receptor (found by some authors) could lead to an acquired stress reactivity [14,17].

Inhibited attachment style was found in all MCI patients, but not in controls. Regarding inhibited attachment in dementia, Magai et al. found demented subjects with an avoidant (inhibited) attachment had higher premorbid levels of contempt and anger or inhibition [23]. Relationships with each parent, and relatives were studied, interviewing on some frequent situation (separation, conflicts, illness, emotional security felt, opportunity to talk about their needs and feelings with parent, or relatives). Subjects presenting inhibited attachment had more difficulty talking to their relatives about their needs. Many avoidance situations with the relative/parent have been noted for many years. They don't talk to their relatives about their own needs and wants, or their worries. Avoidant AS is not only behavioural ("I pretended I didn't care") but also representational in that subjects with inhibited AS are reluctant to admit, think about or genuinely reflect upon attachment-related issues or feelings ("I didn't care anyway"), or maybe they do not want to disturb their parents. They tend to keep their difficulties inside, without talking to relatives about their feelings and needs. Of course, the inhibited attachment is not specific to the MCI group. Finally, we have to analyze these results with caution, given the small and non-representative sample size. Indeed, we have known, according to the literature, that insecure attachment (as a risk factor for affective disorder) is related to anxiety disorders since infancy. Inhibited attachment might be improving the cognitive impairment risk through a not well-being and inadequate emotional life. Finally, we also have found a risk factor such as higher neuroticism in MCI patients, as a premorbid personality trait that has been already found in dementia in a lot of studies [1,2,5]. Inhibited AS could lead to high neuroticism.

LIMITATIONS

We can cite as evidence the following limitations:

* A low number of MCI subjects n=9, also for a pilot study.

* Patients are older than controls.

* Attachment interviews were recorded, but it is very difficult to confirm our results because of retrospective assessment without specific behavioural study, even if the AMMI is validated.

* It would be more difficult to assess attachment in MCI patients, but their autobiographical memory is known to be intact at the beginning of cognitive impairment.

* No OTrx methylation was studied, whereas it could be linked with self-reported attachment avoidance [27]

* Most subjects have lived in infancy during the World War II in Europe that might explain both inhibited and insecure attachment with their parent in that time [28].

CONCLUSION

Despite several limitations, these results are in favour of our hypotheses. We found a link between severe affective BPS, insecure attachment, and the presence of an oxytocin receptor polymorphism (rs 53576; allele A+), but not with the rs2254298 polymorphism which could be involved in anxiety disorders in patients with behavioural inhibited temperament (with

stressful life events in childhood). An inhibited deactivated attachment style was found in all MCI patients. Separation and attachment might be involved in MCI and dementia, with or without BPS [2,3]. Surely; these findings need confirmation in the future. Moreover, we need to achieve a better understanding of the course of the inhibited attachment style in MCI patients. We have also found a well-known risk factor such as higher neuroticism in MCI patients. There might a link between neuroticism and inhibited attachment that might be improving the cognitive impairment risk through a not well-being and inadequate emotional life. However, environmental adversity does not necessary mean that unfavorable effects on development occur postnatally.

Finally, why inhibited attachment profile might be involved in MCI? Neurosciences with Phenomenology could help explain the course of cognitive disorders and dementia [3].

In summary, this overview is in favour of an association of genetic plasticity with both behavioural and neuroanatomical correlates of gene-environment interaction. Moreover, biological aspects may interact with psychological aspects (here inhibited attachment profile in childhood). It needs confirmation in the future. On one hand, our experiment can be used to make the study possible for a larger number of subjects. The methodology appears scientifically rigorous. On the other hand, the need of a longitudinal study has to be done, confirming our goals.

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