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Short Communication

Leukocyte telomere length in mastocytosis: Correlations with depression and perceived stress

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ABSTRACT

Background: Mastocytosisis a rare disease associated with chronic symptoms related to mast cell mediator release. Patients with mastocytosis display high level of negative emotionality such as depression and stress sensibility. Brain mast cells are mainly localized in the diencephalon, which is linked to emotion regulatory systems. Negative emotionality has been shown to be associated with telomere shortening. Taken together these observations led us to hypothesize that mast cells activity could be involved in both negative emotionality and telomere shortening in mastocytosis.
Objective: To demonstrate a possible relationship between negative emotionality in mastocytosis and leukocytes telomere length.
Methods: Leukocyte telomere length and telomerase activity were measured among mastocytosis patients and were correlated with perceived stress and depression assessed by the Beck Depression Inventory revised and the Perceived Stress Scale.
Results: Mild-severe depression scores were frequent (78.9%) as well as high perceived stress (42.11%). Telomere length was correlated to perceived stress ($r = 0.77$; $p = 0.0001$) but not to depression in our population. Patients displaying Wild-type KIT significantly presented higher perceived stress levels.

Abbreviations: CT, control; PBMC, peripheral blood mononuclear cells; RTA, relative telomerase activity.

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Patients with the D816VC KIT mutation who had high perceived stress scores displayed significantly shorter telomere but not if they had high depression scores.
Conclusion: These findings suggest that high perceived stress in mastocytosis could accelerate the rate of leukocytes telomere shortening. Since mastocytosis is, by definition, a mast cell mediated disease; these cells could be involved in this phenomenon. Mechanistic causal relationships between these parameters need to be investigated.

1. Introduction

Mast cells are actors of the innate immune system and play an important role in allergy and anaphylaxis (Gilfillan et al., 2011; Metcalfe, 2008). These cells seem to be involved in a variety of host inflammatory and functional disorders of the lungs, eyes, skin, joints, and gastrointestinal tract (Hamilton et al., 2011). Mast cells accumulation in one or several organs characterizes mastocytosis, a rare and heterogeneous disease. Mastocytosis is usually indolent but in some cases exhibit aggressive features and might be associated to other clonal hematological malignancies (Horny, 2008; Lennert and Parwaresch, 1979; Metcalfe, 1991; Valent et al., 2001a,b). Patients with mastocytosis often present with signs and symptoms that are characteristic of mast cell mediator release. These include flushing, dermatographism, asthma, anaphylaxis, naso-ocular manifestations, neuropsychiatric symptoms (including headache and poor concentration and memory) and gastrointestinal complaints such as abdominal pain, diarrhea (Castells and Austen, 2002; Moura et al., 2011; Rogers et al., 1986; Sokol et al., 2010). Even though patients perceive more disability related to their symptoms than previously thought, their life expectancy is not significantly affected in indolent forms of the disease (Hermine et al., 2008; Lim et al., 2009). Several criteria are necessary for the positive diagnosis of mastocytosis (Akin et al., 2010). Patients with mastocytosis usually display the D816VKIT mutation in the tyrosine kinase receptor c-Kit, resulting in its constitutive activation (Orfao et al., 2007).

In the brain, mast cells are mostly localized in the hypothalamus, thalamus and close to neuronal terminations which are responsible for the adrenocorticortropic hormone (ACTH) release (Edvinsson et al., 1977; Goldschmidt et al., 1984; Johnson and Krenger, 1992; Matsumoto et al., 2001; Theoharides et al., 1995). There is increasing evidence that brain mast cells play a role in mood's changings, cognition, behavior and stress responsiveness (Bienenstock, 2002, 1987; Esposito et al., 2001; Matsumoto et al., 2001; Theoharides, 2009 1990, 1998). We and others have shown that 40–60% of mastocytosis patients present with neuropsychological manifestations including attention and memory impairments and depression (Hermine et al., 2008; Moura et al., 2012, 2011; Rogers et al., 1986). Although the mechanism of depressive symptoms in mastocytosis is not yet understood, a pure psychogenic explanation seems improbable (Hermine et al., 2008; Moura et al., 2011; Rogers et al., 1986) and chronic mast cell activation per se might be involved.

Telomeres are specialized nucleoprotein structures that cap and protect the ends of chromosomes. They can be elongated by the telomerase enzyme. So, in telomerase-negative cells, telomeres shorten after each cellular division (Blackburn, 1991). This phenomenon ultimately leads to cellular senescence, conferring to the telomeres the role of a biological clock (Harley et al., 1990, 1992). Chronic perceived stress has been correlated with shortened telomere length in leukocytes, a phenomenon attributed, in part, to higher levels of oxidative stress at the cellular level (Epel et al., 2004). Furthermore, in the peripheral blood mononuclear cells from patients with systemic diseases, it has been shown that telomeres were short and that telomeric erosion could result from chronic stress exposure (Georgin-Lavialle et al., 2010).

However, the correlation between telomere erosion and depression are not consensual (Hartmann et al., 2010; Hoen et al., 2011; Wolkowitz et al., 2010) probably because of differences in the status of depressive symptoms in the populations studied (endogeneous/secondary depression).

Considering these data, we decided to investigate the links between telomere length and neuropsychological features in mastocytosis. To preliminarily test our hypothesis, we investigated and quantified depression and perceived stress in patients suffering from mastocytosis associated with D816V KIT mutation or wild type (WT) KIT. We correlated these features with disease duration, tryptase levels, leukocyte telomere length and telomerase activity.

2. Materials and methods

2.1. Patients (Table 1)

Adults with a diagnosis of mastocytosis ($n = 19$), as defined by the WHO international consensus criteria (Horny, 2008), were enrolled in a prospective national multicentric French "AFIRMM protocol" study between 2007 and 2011. All patients provided their informed consent. The study was approved by the ethics committee at Necker hospital, and was carried out in compliance with the precepts of the Helsinki protocol. Clinical forms of mastocytosis included benign forms (cutaneous, $n = 2$ and indolent systemic, $n = 13$) and systemic aggressive ($n = 4$). Cutaneous mastocytosis was defined by the excess of pathological mast cells in the skin, but their absence in a bone-marrow or other organs. To establish the diagnosis of mastocytosis, following international WHO criteria, we have provided measurements of tryptase serum level as well as the search of KIT gene mutations in the skin and/or bone marrow as previously described (Bodemer et al., 2010). The study population consisted of 4 men and 15women, with a median age of 45 years (range: 19–78). The KIT D816V mutation was detected in 15 patients, and 4 patients harbored WTKIT. Median serum tryptase level was 25 μg/L (range: 2–280).

2.2. Methods

2.2.1. PBMC analysis

Peripheral blood was obtained by venipuncture in heparinized tubes. PBMCs were isolated using the standard Ficoll–IPaque method (Amersham Life Science, UK). Cells were washed twice in phosphate-buffered saline containing 2% fetal bovine serum. DNA extraction was performed on 5.10^6 cells for telomere-length analysis, and 2.10^5 cells were assessed for telomerase activity.

2.2.2. Telomerase activity

PBMCs (2.10^5) were lysed and a QTRAP assay was performed on protein extract as described by the Allied Biotech® Quantitative Telomerase Detection kit. Measurements were done in triplicate and the mean was calculated. For each sample, the result was expressed as relative telomerase activity after comparison with a

Table 1
Main features of the population study.

Patient number	Sex	Ethny	Age on inclusion (years)	Delay between symptoms and diagnosis	Valent stage	Serum tryptase rate µg/L	*c-Kit* genotype
1	F	Caucasian	50	2	ISM	21	D816V
2	F	Caucasian	36	15	ISM	5.5	D816V
3	M	Caucasian	34	14	ISM	134	WT
4	F	Caucasian	23	3	CM	2	WT
5	F	Caucasian	19	0	ISM	21	WT
6	F	Caucasian	33	1	ASM	30	WT
7	M	Caucasian	48	11	ISM	20	D816V
8	F	Caucasian	48	14	ISM	280	WT
9	F	Caucasian	43	26	ISM	90	D816V
10	F	Caucasian	47	6	ISM	37	D816V
11	M	Caucasian	54	1	ASM	52	D816V
12	F	Caucasian	21	1	ISM	30	D816V
13	F	Caucasian	36	0	ISM	2.7	D816V
14	F	Caucasian	70	18	ISM	15	D816V
15	F	Caucasian	78	3	ASM	25	D816V
16	F	Caucasian	40	2	CM	5	D816V
17	F	Caucasian	76	8	ASM	52	D816V
18	F	Caucasian	37	10	ISM	20	D816V
19	M	Caucasian	52	2	ISM	105	D816V

Abbreviations: M, male; F, female; SM, systemic mastocytosis; ASM, aggressive SM; ISM, indolent SM; CM, cutaneous mastocytosis.

positive control provided by the manufacturer. PBMC's telomerase activity was measured among mastocytosis patients ($n = 12$) and healthy age-matched controls who were age matched healthy adults with neither psychological affection nor mastocytosis. Telomerase activity was not available for 7 patients.

2.2.3. Measurement of telomere length

Genomic DNA was extracted from PBMCs using an Easy DNA kit from Invitrogen®. Telomere length was measured using quantitative real-time polymerase chain reaction (RT-qPCR) on a "Rotor Gene" thermal cycler (Corbett Research, Australia) using DNA extracted from peripheral blood leukocytes. This method has previously been compared with telomere length measurements derived from the mean length of the terminal restriction fragments measured by Southern blot analysis, which is considered the reference (Okayama, 2005). RT-qPCRs were validated. The mean telomere length was measured exactly as described by Cawthon using a sequence of the 36B4 gene (now named RPL0) as the reference (Okayama, 2005). The DNA samples were amplified by qPCR using the Quantifast SYBR Green PCR kit (Qiagen S.A., France). Reaction volumes were 25 µL and PCR cycle parameters were: 94 °C for 15 s, 54 °C for 1 min, and 72 °C for 30 s (35 cycles). Mean telomere length was estimated as the ratio $Ct^{\text{Telomere}}/Ct^{36B4}$, which is inversely proportional to telomere length. Telomere length measurements (TL) in peripheral blood mononuclear cells (PBMCs), as well as psychological assessments, were performed for all mastocytosis patients.

2.2.4. KIT gene sequencing and measurement of serum tryptase

The technique for the KIT gene sequencing has been previously described (Bodemer et al., 2010; Hahn et al., 2008). The level of total tryptase (protryptase + β tryptase) in serum samples was determined using a fluorescence enzyme-linked immunoassay (Unicap; Pharmacia) (Schwartz et al., 1994). The detection limit of the assay was 1 ng/mL and, in healthy controls, serum tryptase levels ranged between <1 and 15 ng/mL, with a median of ~5 ng/mL(Sperr et al., 2001).

2.2.5. Psychological evaluation

Psychological assessment included measures of negative emotionality (depression and stress).Almost all patients (with the exception of 6) could be screened for depression through interview by a trained psychologist. Among patients interviewed ($n = 13$), 69% responded for the criteria for Major Depression as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) ($n = 13$). However, because of the lack of psychological interview for 6 patients we used the Beck Depression Inventory revised (BDI-II) scores which ability for screening depressive disorder has been suggested by several studies (Bunevicius et al., 2012; Lustman et al., 1997; Warmenhoven et al., 2012). In these studies, the optimal cut point of BDI to diagnose depression among patients with somatic disease is between 14 and 16. Though, as we know that self-report-evaluation cannot substitute for the finesse of diagnostic interview we decided to consider a score of at least 19, according to the BDI's manual, to identify patients with moderate-severe symptoms of depression (Beck et al., 1996).The Beck Depression Inventory revised (BDI-II) has been developed according to the diagnostic criteria for Major Depression described in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (Beck et al., 1996). Total score, indicates the severity of participant's depression symptoms: minimal (0–9), mild (10–18), moderate (19–29) or severe (30–63). Thus, according to the BDI-II manual, a cut point of $\geqslant 19$ in BDI was used to identify patients presenting moderate symptoms of depression. Stress was assessed by the Perceived Stress Scale which measures the degree to which life events are appraised as stressful by individuals (Cohen et al., 1983).

2.2.6. Statistical analyses

Statistical analysis of the correlations between the depression, perceived stress and telomere length as well as WHO classification, tryptase level and KIT mutation status were performed controlling for gender and age. In our sample, depression and tryptase were not normally distributed as confirmed by Shapiro–Wilk normality test. The use of Pearson correlation with non-normally distributed data can reduce power and trouble the interpretation. For this reason, we first performed Spearman rank correlation and compared with results obtained using Pearson. Results were very similar regardless of the method used and we chose to present Pearson instead of Spearman correlation coefficients. The correlations analysis was performed with PAWS software version 17.0 (IBM SPSS Statistics Inc., Chicago, USA). All reported p values were two-tailed with a significance level of 0.05. We used GraphPad Prism software version 5.01 (GraphPad Software Inc., San Diego, CA) to perform t-tests. To compare mean of stress, depression and telomere length among groups of patients according to their WHO classification status (aggressive/ non aggressive), their c-kit (wild type vs

D816V) or stress level (high/ low)as well as telomerase activity between patients versus matched controls, we used Unpaired *t*-test.

3. Results

3.1. Patients with mastocytosis have a higher score of depression and perceived stress

The mean score for depression among mastocytosis patients was 19.00 ($SD = 10.30$; range = 7–36). We found that 78.9% ($n = 15$) of patients presented mild to severe symptoms of depression (BDI $\geqslant 10$). Mild depression concerned 36.8% ($n = 7$) (mean = 12.43; $SD = 1.81$, range = 10–15) of patients and moderate-severe depression concerned 42.11% ($n = 8$) (mean = 30; $SD = 5.04$, range = 21–36). High levels of perceived stress (PSS scoring $\geqslant 40$) concerned 47.36% ($n = 9$) of patients (mean = 48.33; SD = 5.65, range = 41–57). After controlling for age and gender, depression was correlated to perceived stress in our sample ($r = 0.586$, $p = 0.014$).

3.2. Wild type KIT gene but not serum tryptase level correlates with perceived stress

There is a significant correlation between KIT mutation and perceived stress ($r = -0.610$, $p = 0.009$). Wild-type KIT ($n = 4$) patients significantly presented with higher perceived stress levels ($t = 3.87$, $p = 0.001$) but no difference was found in regards of depression ($t = 0.48$, $p = 0.642$) or telomeres length ($t = 1.75$, $p = 0.092$). KIT mutation was not correlated to depression ($r = -0.428$, $p = 0.086$) or to telomere length ($r = 0.409$, $p = 0.103$).

Serum tryptase levels were not correlated to depression ($r = 0.147$, $p = 0.573$), perceived stress ($r = 0.040$, $p = 0.880$) or telomere length ($r = -0.036$, $p = 0.889$). WHO classification (Valent et al., 2001a,b) status was not correlated to depression ($r = 0.380$, $p = 0.133$), perceived stress ($r = 0.110$, $p = 0.674$) or telomere length ($r = 0.112$, $p = 0.668$). Only four patients were concerned with aggressive mastocytosis but no difference was found with respect to depression, perceived stress or telomere length (ps = 0.902–0.637).

3.3. Telomere were shortened among patients with high perceived stress

Telomere length was correlated to perceived stress ($r = -0.728$; $p = 0.001$) in our population (Fig. 1A) even after controlling for depression ($r = -0.661$; $p = 0.005$). When comparing patients presenting high level of perceived stress ($n = 9$, scores $\geqslant 40$) (mean = 48.33, $SD = 5.65$ range = 41–57), versus patients with low level of perceived stress ($n = 10$) (mean = 34, $SD = 2.64$, range = 28–37), we found that the first group has shorter telomeres (mean = 0.049, $SD = 0.03$, range = 0.44–0.52 versus mean = 0.57, SD = 0.07 range = 0.44–0.70) ($t = 3.28$, $p = 0.004$) and higher depression scores (mean = 25, $SD = 10.02$ range = 7–36 versus mean = 14, $SD = 8.03$, range = 9–36) ($t = 2.55$, $p = 0.021$) (data not shown). Furthermore, in D816V KIT mutation patients group ($n = 15$), there was a significant difference of telomere length between patients with high or low perceived stress scores ($p = 0.0375$) (Fig. 1C).

3.4. Telomere length was not linked to depression among patients

Telomere length was not correlated to depression in our population ($r = -0.407$; $p = 0.105$) (Fig. 1B). Even if focusing on patients with the D816V KIT mutation ($n = 15$), there was no difference of telomere length between patients with high depression scores and patients with low depression scores ($p = 0.322$) (Fig. 1D).

3.5. Telomerase activity was not higher among patients with mastocytosis

In order to study if short telomere length could induce reactivation of telomerase, we measured the relative telomerase activity in the peripheral blood mononuclear cells of mastocytosis patients compared to age-matched healthy controls. This activity was not different between patients and controls ($p = 0.149$) (Fig. 1E).

4. Discussion

This is the first study showing that perceived stress even after controlling for depression, is significantly correlated to shorter telomere length in the leukocytes of patients with mastocytosis. Moreover, on average, patients with high perceived stress presented more severe depressive symptoms and shorter telomeres. Neither tryptase level, nor mastocytosis WHO status, nor KIT mutation, were correlated to leukocytes telomere length. Among patients with the D816V KIT mutation, short telomeres were significantly associated with higher level of perceived stress but not with depression. Patients with WT KIT presented higher level of perceived stress than patients with D816V KIT mutation, but no difference was found with respect to depression or telomere length. Due to the small numbers of patients, no definitive conclusion can be drawn and confirmatory studies in aggressive and indolent forms of mastocytosis concerning depression level, perceived stress level or telomere length are needed.

Telomere shortening has previously been reported to be associated with psychological stress (Epel et al., 2004) but the relations to depression are not consensual (Wolkowitz et al., 2010; Shaffer et al., 2012).We found high level of perceived stress among these patients which is in line with our previous work reporting that patients with mastocytosis display high level of depression and anxiety (Moura et al., 2012, 2011). Interestingly, among mastocytosis patients, we report for the first time, increased telomere erosion in leukocytes especially among patients with high perceived stress. The telomere shortening was not adequately compensated by increased telomerase activity, which was the same between patients and controls. This could suggest either accelerated leukocytes aging without subsequent increased telomerase activity to stabilize or lengthen telomere, or it could reflect increased leukocytes senescence.

A reduced leukocyte lifespan could result from increased oxidative cellular stress in response to psychological distress. To explain this phenomenon, it has been hypothesized that psychological stress can induce oxidative cellular stress, which may lead to telomere shortening and reduce cellular lifespan, if telomerase is not reactivated (Epel et al., 2004, 2009). However, the link between brain and leukocytes remains unknown. Interestingly, mast cells can produce reactive oxidative species (ROS) upon various stimuli independently of IgE stimulation, and it could be speculated that neurological stimuli could be one of these stimuli (Okayama, 2005; Wolfreys and Oliveira, 1997). Thus, in agreement with our hypothesis, mast cells and their mediators could play a critical role at the interface between brain and circulating leukocytes.

Our patients with mastocytosis presented with significant levels of perceived stress and depression. Patients with indolent forms do not develop the major physical symptoms of mastocytosis and their life expectancy is similar to general population (Lim et al., 2009). Interestingly, the WHO stage of disease, indolent versus aggressive, was not correlated with depression and perceived stress in our sample. This result is important and shows that pa-

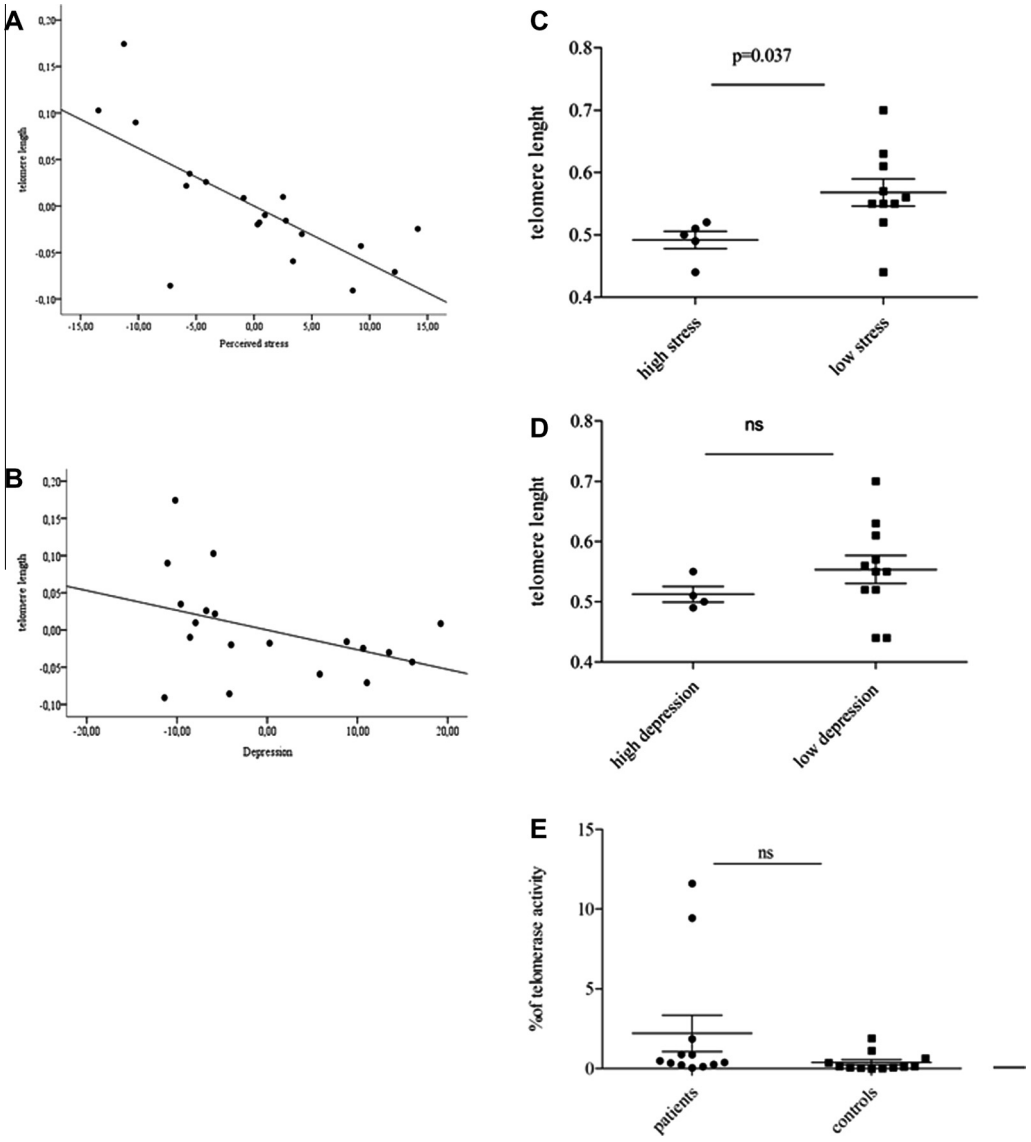

Fig. 1. (Panels A and B) Correlations between telomere length and psychological variables. (A) and (B) show correlations between telomere and psychological variables controlling for age and gender. Telomere length was strongly correlated to perceived stress ($r = -0.728$; $p = 0.001$) (A) but not to depression ($r = -0.407$; $p = 0.105$) (B). (Panel C) Patients with D816Vc-KIT mutation and high perceived stress displayer shorter telomere ($t = 2.317$, $p = 0.037$). (Panel D) Patients with D816Vc-KIT mutation and high depression scores did not display short telomere ($t = 1.029$, $p = 0.322$). (Panel E) Comparison of leukocyte telomerase activity between patients and controls. We compared the leukocytes relative telomerase activity, measured by QTRAP in 12 patients with mastocytosis and 12 age-matched healthy controls. The relative telomerase activity was not different between mastocytosis patients and controls ($t = 1.549$, $p = 0.149$).

tients with aggressive mastocytosis are not the most distressed patients. Thus, the significant distress levels found among these patients cannot be explained by "chronic disease-associated distress" confirming previous studies, which suggest that the psychological symptoms of mastocytosis patients are not related to disease severity, but probably more to the activation of mast cells (Hermine et al., 2008; Rogers et al., 1986). Our recent study, which showed a major effect of Masitinib, tyrosine kinase inhibitor, targeting c-Kit, on reducing depression scores of these patients, suggests that psychological symptoms in mastocytosis are primary symptoms related to mast cell activity rather than secondary symptoms that have developed as a reaction to the physical impact of the disease (Moura et al., 2011; Paul et al., 2010). Here we found higher perceived stress among patients with WT KIT compared to patients with D816V KIT mutation. It is difficult to conclude on this finding because few patients were involved but negative emotion susceptibility could be a specific feature of mastocytosis patients without the classical D816V KIT mutation. It is tempting to speculate that molecular mechanisms responsible of mastocytosis in WT KIT patients may induce a different pattern of mediator release. These results could be confirmed on a larger cohort of mastocytosis patients with complete KIT sequencing as well as full psychological evaluation.

Consistent with other studies, perceived stress was correlated with shortened telomere length (Epel et al., 2004, 2009; O'Donovan et al., 2009; Simon et al., 2006). Herein, we have shown, for the first time, correlations within a population with mast cell-mediated disease. Interestingly, despite the small size of our cohort, the relationship between perceived stress and telomere length was very strong. Given observed associations between mastocytosis, high perceived stress and shortened telomere, it is tempting to hypothesize that mast cells could be responsible for psychological manifestations in mastocytosis through high negative emotions/stress susceptibility and exposure to psychological stress could lead to telomere shortening across the lifespan.

Mast cells could also be implicated in telomere shortening via a specific mast-cell mediated pathway. This hypothesis is supported by different studies describing the implication of mast cells in both limbic and stress systems. The corticotropin-releasing hormone (CRH) is produced by the hypothalamus and is the main coordinator of the stress response through the activation of the limbic system, the hypothalamic–adenohypophisaire and sympathetic nervous system. In dogs, it has been shown that mast cells could evoke a response of the hypothalamic–adenohypophisaire (HPA) in response to IgE and FcεRI expression (Matsumoto et al., 2001). Stress activates the mast cell in the brain, particularly those close to the nerve fibers responsible for the release of adenocorticotrophine (ACTH) (Theoharides et al., 1995) and increases the permeability of the blood–brain barrier via the activation of these cells (Esposito et al., 2001).

The specific high stress susceptibility to stress of mastocytosis patients could sign the high implication of mast-cells in both emotional and physiological stress responsiveness in this disease. Further research should investigate the psychological and physiological stress responsiveness of mastocytosis patients. Particularly those concerned with WT KIT which seems to present higher perceived stress susceptibility. Mastocytosis could be accompanied by a "chronic hyper-activation" of emotion and physiological stress responses mechanisms in brain which could explain the extremely higher scores of negative emotion in this population and at the same time suggest that oxidative cellular stress could be induced by mast cells through chronic activation of physiological stress responsiveness. In this work, we assessed depression using the revised Beck Depression Inventory and not a diagnostic interview. However, the ability of the Beck Depression Inventory for screening depressive disorder has been suggested by several studies and seems well established (Bunevicius et al., 2012; Lustman et al., 1997; Warmenhoven et al., 2012). In these studies, the optimal cut-point of BDI to diagnosing depression among patients with somatic disease is between 14 and 16. Therefore, we decided to consider a higher cut-off of at least 19. Even though variability in our results, due to the type of method used to detect depression and the limited sample size, our results call for investigations on the role of mast cells in both emotionality and cellular aging. Further research will be needed to study the relationship between psychological distress and mast cells and to assess causality and, particularly, mastocytosis being a model of mast cell chronic activation.

In conclusion, patients with mastocytosis present high levels of depression and perceived stress. The leukocytes telomere length of these patients was significantly shorter if they presented high perceived stress but no relation was found concerning depression. These results suggest that mast cells infiltration and/or activation in mastocytosis could be responsible for the abnormalities detected in the telomere–telomerase system in this pathology. Further researches are necessary to investigate mast cells implication in cell senescence and brain. Mast cell could be a possible target to treat psychiatric disorders and to prevent consequence of telomere shortening associated to these conditions.

Conflict of interest statement

All authors declare that there are no conflicts of interest.

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