




Brain-Derived Neurotrophic Factor (BDNF) and Serotonin Transporter (SERT) in Platelets of Patients with Mild Huntington's Disease: Relationships with Social Cognition Symptoms

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Abstract. Peripheral biological correlates of early-stage Huntington's disease (HD) are currently attracting much interest given their possible use as prognostic predictors of later neurodegeneration. Since deficits in social-cognition processing are present among the initial disease symptoms, aim of this work was to appraise, in blood platelets, Brain-Derived Neurotrophic Factor (BDNF) and serotonin (5-HT) transporter (SERT), two proteins involved in human adaptive behavior as potential biochemical correlates of such disabilities in mild-HD.

Thirteen gene positive and symptomatic patients (9M/4W, HD-stage II, age > 40y) together 11 gender/age matched controls without a concurrent diagnosis of psychiatric disorders, underwent a blood test to determine BDNF storage and membrane-bound SERT in platelets by ELISA immune-enzyme and [³H]-paroxetine ([³H]-PAR) binding assays, respectively. Concomitantly, all subjects were examined through a battery of socio-cognitive and emotion recognition questionnaires.

Results showed moderately increased intra-platelet BDNF amounts (+20-22%) in patients versus controls, whereas [³H]-PAR binding parameters, maximum density (B_{max}) and dissociation constant (K_D), did not appreciably vary between the two groups. While patients displaying significantly reduced cognitive/emotion abilities, biochemical parameters and clinical features or psychosocial scores did not correlate each other, except for platelet BDNF and the illness duration, positively correlated, or for SERT K_{DS} and angry voice recognition ability, negatively correlated in both controls and patients. Therefore, in this pilot investigation, platelet BDNF and SERT did not specifically underlie psychosocial deficits in stage II-HD. Higher platelet BDNF storage in patients showing lasting-mild symptoms would derive from compensatory mechanisms. Thus, supplementary investigations are warranted by also comparing patients in other illness's phases.

Keywords: Huntington's disease, Social Cognition, Platelets, Brain-Derived Neurotrophic Factor, Serotonin transporter.

Introduction

Huntington's disease (HD) is a rare neurodegenerative illness caused by an autosomal dominant gene mutation. Next to the typical motor disturbances, identified by choréic uncontrollable movements, HD is also defined by a constellation of cognitive, psychiatric, behavioral and even peripheral signs (Zuccato and Cattaneo, 2014; Schiefer et al, 2015), often resulting in the misdiagnosis and problematic follow up of patients. In the pre-symptomatic and initial phases, prevailing HD signs are subtle changes of mood-anxiety tonus, apathy, unreliability at work, aggressive behavior, forgetfulness and reduction of multitasking performance accompanied by mild motor symptoms as restlessness and disturbed motor coordination (Ross et al, 2014; Schiefer et al, 2015, Waldvogel et al, 2015). Conversely, later stages are characterized rather by a gradual worsening of motor functions and cognitive decline until demise, occurring on average 20 years after the illness onset (Zuccato and Cattaneo, 2014). Between these two phases, intermediate stages of progression (Shoulson and Fahn, 1979) classify the highly variable phenotypes of HD (Waldvogel et al, 2015).

At the biological standpoint, the mutation responsible of HD is the abnormal expansion of CAG triplets corresponding to the amino acid glutamine (Glu, Q) in the gene HTT (IT15) encoding the protein huntingtin (Htt) on the short-arm of human chromosome 4 (Finkbeiner, 2015). The wild-type WT-Htt poly-Glu expansion is located at the N-terminal domain of the protein and consists in a 10-30 Glu repeat. Glu repeats ≥ 36 are associated instead with symptoms' arise and their length has been found negatively correlated with the age of illness onset (Penney et al, 1997). Neurodegenerative processes in HD can be promoted by the loss of WT-Htt function owing to the misfolding of mutant-Htt (*m-Htt*) and/or by the gain of toxic functions deriving from the expression of this last (Bates, 2003; Zuccato and Cattaneo, 2014). Both mechanisms are supposed to promote the gradual apoptosis of GABAergic medium-sized spiny neurons localized in basal ganglia and striatum (Halliday et al, 1998; Ross et al., 2014), extended to neuronal populations belonging to other brain areas during the evolution of

neurodegenerative processes, as revealed by brain imaging and post-mortem studies (Ross and Tabrizi, 2011). Although researches on both WT-Htt protective roles and *m*-Htt-related cytotoxic effects are taking place with success, noteworthy many pathogenic aspects of the disease's progression remain scarcely understood, especially as concerns the selective vulnerability to the genetic mutation, both at the cellular and molecular levels. It is worth noting that neurotransmitters as dopamine and glutamate (Jakel and Maragos, 2000; Han et al, 2010) or neuropeptides as enkephalins, substance P and dinorphins (Holt et al, 1997; Morfini et al, 2005; Han et al, 2010) have been linked to the selective neuronal susceptibility to *m*-Htt in HD.

Additionally, next to animal studies and human neuroimaging or post-mortem investigations, focused on the brain pathogenetical features of the illness, increasing attention is dedicated to the search of suitable peripheral biomarkers and metabolic predictors, using more accessible biological samples, as plasma, serum, saliva and blood cells. These are expected to be much helpful for the monitoring of HD development and “aggressiveness” (Ross and Tabrizi, 2011; Weir et al, 2011; Ross et al, 2014). Indeed, a main aspect needing elucidation is precisely the link between distinct phases/symptoms, altered central/peripheral biological factors and ongoing neuronal death: the early identification of clinical and biochemical profiles in HD would implement more targeted therapeutic approaches to delay evolution towards terminal phases (Ross et al, 2014).

Among biological matrices, blood platelets are in particular considered an attractive target for investigations on psychiatric and neurodegenerative diseases, since they are a kind of “window to the brain”, containing enzymes, carrier proteins, NTs, neurotransmitters, and receptors active in CNS and synapses (Stahl, 1977; Da Prada et al, 1988; Camacho and Dimsdale, 2000; Asor and Ben-Shachar, 2012; Erlich and Humpel, 2012; Yubero-Lahoz et al, 2013; Hayashi-Takagi et al, 2016; Lai et al, 2016). Moreover, numerous evidences highlight the existence of relevant networks/cross-talks between the brain and the bloodstream (Felger and Lotrich, 2013; Carvalho De Fonseca et al, 2014; Pfau and Russo, 2015).

For what concerns instead peculiar symptoms observed in HD patients, relevant inabilities in emotion perception and empathy functions (Jauhar and Richie, 2010) have been identified. Some authors have proposed that such socio-cognitive and emotion recognition deficits (Ille et al., 2011; Henley et al., 2012), may be indexes of HD evolution in the direction of more severe brain injuries (Bora et al, 2016). Neurocognitive impairment in HD not only includes deficits in abilities assessed by traditional neuropsychological batteries such as memory and executive functioning (Peavy et al, 2010), but also often features social cognition deficits which encompasses how individuals perceive and respond to social situations.

One aspect of social cognition, the recognition of emotion from faces, has been extensively studied in the last 20 year. Particularly, as far as disgust is concerned, a widespread recognition deficit of emotion, also in response to other stimuli such as vocal and body language, has been evidenced, thus suggesting the presence of a prevalent impairment that affects negative emotions (Henley et al, 2012).

Another feature of social cognition is theory of mind (ToM), which is the ability to attribute mental states (feelings, beliefs, intentions, and desires) to others, in substance to understand and predict others' behavior based on their mental states (Frith and Frith, 2012). ToM is not an entirely a homogeneous concept, and some authors have suggested that ToM includes both affective and cognitive components, involved in reasoning vs. decoding of mental states in HD patients (Adjeroud et al, 2016).

Both ToM and emotion recognition abilities are critical for adaptive and effective social functioning. ToM and emotion recognition deficits are likely to be clinically relevant, as difficulties in social interaction, communication and poor insight frequently occur in HD (Adjeroud et al, 2016).

In such context, the present pilot study aimed to measure, compare and possibly relay the psycho-cognitive abilities of a small but stage homogeneous group of mild symptomatic HD patients (stage II, Shoulson and Fahn, 1979) and matched controls, with two proteins implicated in human behavior and emotion processing, the neurotrophin (NT) Brain-Derived Neurotrophic Factor (BDNF) (Murer et al,

2001; Autry and Monteggia, 2012; Homberg et al, 2014) and SERT, the reuptake site of the neurotransmitter serotonin (5-HT) (Hariri et al, 2002; Ozaki et al., 2003; Canli and Lesch, 2007; Lesch, 2007). Briefly, subjects, selected on the basis of the Unified Huntington's Disease Rating Scale (UHDRS) for motor symptoms, were investigated through a battery of questionnaires to evaluate their cognitive skills and, accordingly to ToM, their empathy and psychosocial abilities. BDNF and SERT were also measured in patients and controls, using, as afore indicated, the non-invasive and non-neuronal peripheral model of blood platelets.

Methods

Chemicals

[³H]-paroxetine (specific activity: 15.5 Ci/mmol) was purchased from Perkin-Elmer, Life Science, Milan, Italy. All other reagents employed in the study were of the best analytical grade and purchased as indicated above.

Subjects

Thirteen patients (4 females, 9 males) and 11 age and sex-matched controls (3 females, 8 males) were enrolled in this study. All subjects were recruited by expert neurologists at the Movement Disorder Center of the Neurology Unit, Department of Clinical and Experimental Medicine, University of Pisa. Patients were clinically classified according to the “Unified Huntington's Disease Rating Scale” (UHDRS by the Huntington Study Group) (Shoulson and Fahn, 1979, Siesling et al, 1998). Patients' inclusion criteria were: age > 40 years, a diagnosis of HD confirmed by UHDRS scores and genetic tests, a good health condition evaluated by a physical examination; exclusion criteria were: presence of pain, sleep/psychiatric disorders and relevant cognitive decline, presence of cancer, cardiovascular diseases, diabetes, liver, kidney or lung diseases, alcohol or drug abuse. Control subjects were recruited among outpatients of the Neurology Unit, healing from neurological disorders not affecting CNS.

Controls' inclusion criteria were: age > 40 years, normal physical and CNS neurological examinations, UHDRS scores under the cut-off, negative familial anamnesis for HD. Controls' exclusion criteria were: presence of pain, sleep/ psychiatric disorders and relevant cognitive decline, presence of cardiovascular diseases, diabetes, liver, kidney or lung diseases and alcohol or drug abuse. No recruited subject was obese, showing a body mass index (BMI) $\leq 25 \text{ Kg m}^{-2}$ (Giannaccini et al, 2013). At the time of enrollment, patients were receiving the lowest effective drug dosages of tetrabenazine, amantadine or pramipexole alone (n=4) or in combination with other medications (n=8): olanzapine (n=1), sodium valproate/pregabalin (n=5), antidepressants as venlafaxine, mirtazapine or trazodone (n=3) and Selective 5-HT Reuptake Inhibitors (SSRIs, n=3). One patient was taking trazodone in monotherapy. Antidepressants, antipsychotics or mood stabilizers were administered at dosages never attaining the psychiatric ones. In any case, prior to evaluation, patients underwent a drug wash-out period of 7 days, the minimum allowed for ethical reasons. Control subjects were drug-free for at least 1 month at the time of enrollment. All patients and controls have read, agreed and signed a written informed consent to participate to the study, previously approved by the Ethical Committee of the University of Pisa.

Cognitive and Psychosocial Evaluation

Next to the neurological examination and the UHDRS scale for motor symptoms, enrolled subjects were investigated by two kinds of questionnaires, administered in sequence. The same day of blood collection, all subjects underwent a sequence of cognitive and psychosocial tests: a first battery of questionnaires consisted in a cognitive evaluation by means of: the Mini Mental State Examination (MMSE) for global cognitive state (Folstein et al, 1975); the Montreal Cognitive Assessment (MoCA) (Nasreddine et al, 2005); the Frontal Assessment Battery (FAB) on mental flexibility, motor programming, sensitivity to interferences, inhibitory control and environment autonomy (Dubois et al, 2000); the Short-term Intelligence Test (TiB) (Sartori et al, 1997) on intelligence and reading abilities. The second battery of tests was a series of psychological and socio-cognitive surveys, accordingly to ToM (Frith and Frith, 2012): the "faux-pas task" test and its sub-scales (Fp/Fp, Fp/c, n/Fp, n/c) (Stone et al., 1998); the test for the ability to perceive faces and emotions according to the Karolinska Directed

Emotional Faces (KDEF) (Lundkvist et al, 1998); the test of emotions attribution after a verbal trigger, VE and VE/sadness (Prior et al, 2003) ; the empathy “Strange Stories” test (Joliffe and Baron-Cohen, 1999; Prior et al, 2003); the “Wilhelm Busch vignettes” test (Inoue et al, 2004). All these questionnaires were performed and evaluated by a skilled psychologist and qualified neurologists of the Neurology Unit of the Department of Clinical and Experimental Medicine of the University of Pisa.

Blood Collection and Platelet Separation

Blood samplings were always carried out during a collection for routine laboratory tests. Fifteen ml of peripheral venous blood were drawn from fasting subjects in clinostat position between 8.00 and 9.00 a.m. at the division of Neurology, University of Pisa, and collected into vacutainer tubes containing the anticoagulant EDTA (1mg ml⁻¹). After collection, tubes were kindly mixed and blood immediately transferred into Falcon tubes containing the protease inhibitor aprotinin (0.1 mg ml⁻¹, Sigma Aldrich). Within 30 min from collection, samples were centrifuged at low-speed (150 g) for 20 min at 20°C to separate the platelet rich plasma (PRP). Platelets were then precipitated from PRP by an ensuing centrifugation at 1,500 g for 15 min at 20°C, followed by a washing step. Resulting pellets, previously divided in 2 aliquots, were stored at -80°C until assay.

Intra-platelet BDNF Determination

The day of assay, one aliquot of platelet pellets stored at -80°C was thawed and used for the evaluation of the BDNF content. To attain this aim, platelet soluble fractions were separated. Briefly, platelets were put on ice and suspended in 7.5 ml of lysis buffer containing 10 mM Tris-HCl, pH=8, and a mix of protease inhibitors (Sigma Aldrich, Protease Inhibitor Cocktail, cod: P8340) at a final dilution of 1:1500 (v:v). Platelets were then homogenized by sonication for about 60 sec. using an ultrasonic sonicator (Sonics Vibracell). The ensuing homogenate was transferred in eppendorf tubes and centrifuged at the maximal velocity for 8 min by a microfuge. Supernatants containing the platelets’ soluble fraction were first measured for their protein content by means of the Bradford method (Bio-Rad), using γ -globulins as the standard. BDNF was then determined by means of a commercial enzyme-linked immunosorbent

assay (ELISA) kit (Promega, Emax ® solid-phase ImmunoAssay System, Wallisellen, Switzerland), after suitable dilutions of platelet soluble fractions, established according to the method's linearity. After following the entire kit procedure, BDNF was measured in all samples using a Wallac Victor2 multilabel/multitask 96-wells plate reader (PerkinElmer, USA), preset at 450 nm. Intra-platelet BDNF content was interpolated as pg ml^{-1} from the kit calibration line. The method was enough sensitive for the study, showing limits of quantification as low as $\text{BDNF} = 15 \text{ pg ml}^{-1}$. Owing to the considerable inter-individual variance of the number of circulating platelets, BDNF levels were normalized for the total protein amount (mg ml^{-1}) of platelet soluble fractions. Prior to statistical investigations, the NT levels were thus converted from pg ml^{-1} into pg mg^{-1} protein.

[³H]-Paroxetine Binding Assay for SERT Determination

Platelet membrane preparations and [³H]-paroxetine ([³H]-PAR) binding assays were carried out following a previously described procedure (Giannaccini et al, 2013). In brief, at the time of the assay, one aliquot of washed platelet pellets was thawed and re-suspended in 10 volumes (w:v) of ice-cold 5 mM Tris-HCl buffer (pH 7.4), containing 5 mM EDTA and protease inhibitors (benzamidine 160 $\mu\text{g/ml}$, bacitracine 200 $\mu\text{g/ml}$; trypsin soy inhibitor 20 $\mu\text{g/ml}$). After homogenization by Ultraturrax, samples were centrifuged at 22,500 g for 15 minutes at 4°C. The resulting membrane-containing pellets were suspended in 10 volumes (w:v) ice-cold 50 mM Tris-HCl buffer (pH 7.4) and washed twice by centrifugation at 48,000 g for 10 minutes at 4°C. The final membrane pellets were suspended in the assay buffer, a 50 mM Tris-HCl buffer (pH 7.4), containing 120 mM NaCl and 5 mM KCl. Protein content was determined by the Bradford's method, as above indicated.

The SERT binding parameters, the maximal binding capacity, (B_{max} , fmol mg^{-1} protein) and the dissociation constant (K_{D} , nM), were evaluated in platelet membranes by measuring the specific binding of the selective radioligand [³H]-PAR (Giannaccini et al, 2013). Saturation experiments were carried out as follows: 100 μl of membranes (50–100 μg proteins) were incubated in the assay buffer with five increasing concentrations of [³H]-PAR, from 0.08 to 1.5 nM in a final volume of 2 ml. Non-specific

binding was appraised in the presence of 10 μ M fluoxetine, as the cold displacer. Incubation was performed for 60 min at 22-24°C and halted by rapid filtration using Wathman GF/C glass fiber filters with a Brandell filtration apparatus. Filters were then washed three times with 5 ml ice-cold buffer assay, put into pony vials and measured for radioactivity (dpm) through a liquid phase scintillation β -counter (Packard 1600 TR). Specific binding was obtained by subtracting, from total binding, the residual (non-specific) binding in the presence of 10 μ M fluoxetine: $B_{sp} = B_{tot} - B_{Nsp}$.

Calculations and Statistics

Data are presented as the mean \pm Standard Error of the Mean (S.E.M). Equilibrium-saturation binding parameters, the B_{max} (fmol/mg protein) and the K_D (nM), were calculated from specific binding through the iterative curve-fitting computer software EBDA-LIGAND (Kell for Windows, v. 6.0) and the GraphPad Prism program (version 5, San Diego, CA, USA). The [3 H]-PAR B_{max} represents the degree of SERT expression (SERT density) on platelet membranes, whilst the equilibrium-binding constant K_D , is inversely related to the transporter affinity for the ligand. Inferential statistical analyses comprised non-parametrical Mann-Whitney U-test, used for comparisons concerning clinical test scores, as well as Student *t*-tests, used for between-group comparisons regarding biochemical results. Pearson correlations were carried out to possibly find correlations between clinical scores and BDNF or SERT parameters.

Descriptive or inferential statistics and any other data analysis were conducted using the Graph-Pad Prism software (version 5.0, San Diego, CA, USA). The ‘box-plot and whiskers’ graphical method (Chambers et al, 1983) was employed to evaluate the possible presence of outlier data. A 2-sided P-value of .05 was considered the statistical threshold.

Results

Subjects

The demographic characteristics of all recruited subjects are shown in Table 1, whilst Table 2 reports the main clinical features of HD patients at the time of their recruitment. Enrolled patients had heterozygote genotypes for the mutant HTT gene, showing moderate allele CAG expansions. Patients were all classified in a stage II of the illness, accordingly to the Shoulson and Fahn criteria (1979). One patient could not complete the cognitive/psycho-social interviews after blood collection and abandoned the study. Therefore, biochemical evaluations were conducted including this patient who interrupted the study, after his former consent, while clinical evaluations were performed using data from the remaining 12 patients.

Cognitive and Psychosocial Evaluation

Patients showed significantly reduced performances in many of the cognitive and psychological tests by means of Mann-Whitney analysis in respect to control subjects. As shown in Table 3, patients displayed significantly lower scores at the MoCa, FAB and “faux-pas” tests ($P < .01$) than controls. Moreover, patients showed reduced abilities in emotion recognition (KDEF, $P < .05$), exhibiting increased difficulty at perceiving fear faces ($P < .01$) or emotions after a verbal trigger (VE, $P < .01$). Patients showed also a reduced ability at the VE/sadness test ($P < .05$). When considering patients and controls altogether ($n = 23$) for correlation tests, younger and more school-educated subjects displayed better scores in the ability at recognizing anger, disgust and sadness (negative emotions) (Spearman, $r(\text{age}) = -0.55$, $P < .05$; $r(\text{schooling}) = 0.7$, $P < .01$). Comparable results were obtained when the two groups of subjects were evaluated separately. Therefore, we report herein reduced psycho-cognitive abilities of HD patients quite in agreement with previous works (Ille et al., 2011; Henley et al., 2012; Bora et 2016), while observing an influence of individual variables as ageing and scholar education on emotion recognition skills.

Comparisons between HD Patients and Controls for Intra-platelet BDNF and [³H]-Paroxetine

Binding:

Figure 1 depicts the BDNF results obtained in all patients and all control subjects: higher levels (on average +22%), close to the significance threshold, were reported in patients than in controls (t -test, $.05 \leq P \leq .075$). All examined controls had platelet BDNF values below 3,000 pg mg⁻¹ protein, the patients' mean level, except for 2 subjects. By examining data through the 'box-plot and whiskers' method, one value was found to exceed the upper interquartile (IQ) in the control group only: after excluding this value, the ensuing t -test resulted significant ($P=.011$).

Instead, pertaining to platelet SERT binding parameters evaluated by [³H]-PAR, no outlier value was found. Also, neither SERT number (B_{max}) nor affinity (inverse of K_D) were found to significantly vary in patients vs. controls: as shown in Figure 2a,b, mean B_{max} or K_D values were found reduced and increased, respectively, without reaching however the statistical significance (t -test, $P > .05$).

Correlations with Psychosocial Abilities and HD Clinical Scores for Intra-platelet BDNF and [³H]-Paroxetine Binding:

No significant correlation between platelet BDNF levels and scores obtained through cognitive or emotion recognition tests was reported ($P > .05$); in patients, intra-platelet BDNF was found also unrelated to UHDRS scores, age, school education and age of HD onset ($P > .05$). This parameter was positively correlated with HD duration only ($r=0.6$; $P < .05$, Figure 3a): patients in early phases of HD since a longer time were also those showing the highest contents of BDNF. As concerns correlations of [³H]-PAR binding, no significant relationship between SERT density (B_{max}) and HD/psycho-cognitive scores was obtained. Conversely, [³H]-PAR K_D s were found correlated to emotion recognition skills: a lower SERT affinity (greater K_D) corresponded to a decreased ability at the emotion recognition test after a verbal trigger ($r=-0.48$; $P=.028$), in particular after verbal anger ($r=-0.60$; $P = .006$) in patients and control subjects altogether. When correlations were conducted in patients and controls separately, the statistical significance was maintained; the significant correlation between K_D and verbal anger

recognition test obtained in patients is shown in Fig. 3b ($r=-0,7$; $P = .013$). Moreover, this correlation was still significant by excluding patients taking antidepressant drugs ($n=9$, $r=-0.68$; $P=.02$).

Discussion

Firstly, even though limited by the small sample size, the present study reports results in line with data literature concerning neuropsychological evaluations, showing a deficit in cognitive and executive functions in patients compared to controls, as shown by MoCa and FAB scores. Moreover, patients showed difficulty in recognitions of negative emotions, independently of the stimuli (faces, verbal), as demonstrated by Henley et al, 2012.

In respect, instead, to BDNF comparison and correlation results in our groups of subjects, an increased BDNF (more than 20%) was found inside platelets of patients vs. controls, but NT intra-platelet content was independent from socio-cognitive signs of stage-II HD or control subjects. Platelet BDNF positively correlated with the disease's duration only. Increased amounts of platelet BDNF in HD are not easily explainable. Indeed, BDNF has been found reduced consistently in the brain of HD patients, even in early phases (Zuccato et al, 2008; Zuccato and Cattaneo, 2009; Zuccato et al., 2011), and its blood levels are supposed to reflect the central contents (Klein et al, 2011). On the other hand, this result was not replicated in serum, plasma or whole blood of HD patients, owing to methodological problems interfering with the accuracy of data (Zuccato et al, 2011). In our study, to circumvent such problems, we processed EDTA and aprotinin-treated blood always within 30 min after collection to restrain variability and artifacts caused by sampling procedure. To explain our results, we thus considered that, in some former investigations, fluctuations and overall raise of serum BDNF have been reported in early stages of other neurodegenerative diseases (Laske et al, 2006; Angelucci et al, 2010; Diniz and Teixeira, 2011; Ventriglia et al, 2013). Thus, accordingly to the concept that serum BDNF contents comprise the amount released by platelets during blood clotting plus plasma levels (Lommatzsch et al, 2005), the increase of intra-platelet BDNF observed herein would represent a compensatory attempt to counteract

central losses in early neurodegeneration phases, as stage-II HD. Under this perspective, an augmented intra-platelet BDNF positively correlated with the illness duration could be due to delayed terminal symptoms and/or a more prolonged exposure to neuroprotective drugs. Augmented platelet BDNF could be for instance associated to the activation of counter-regulatory redox signaling/responses (Facheris et al. 2004; Li et al, 2013). Emerging aspects in neurodegenerative processes have been indeed identified at the crossroad between redox homeostasis, tryptophan metabolism as well as inflammation paths and mitochondrial function (Sas et al, 2007; Shukla et al, 2011). Noteworthy, an increased platelet responsiveness to aggregation stimuli was observed in symptomatic patients (Muramatsu et al, 1970) together a blunted NO release in advanced HD (Carrizzo et al, 2014). It seems therefore plausible to assume that platelets change their reactivity in HD, showing BDNF contents that fluctuate dynamically as a function of the progression of the disease and/or administered treatments. Nonetheless, before drawing any definitive conclusion, procedural issues intrinsic to the measurement of platelet BDNF or biochemical aspects linked to the regulation of circulating BDNF expression could still prevent to obtain robust results. A methodological improvement would be thus necessary, by overall considering platelet reactivity, the different degree of NT maturation and pro-BDNF levels, bound or free forms, as well as the NT transport and compartmentalization rate inside platelet α -granules to attain its balanced storage (Tamura et al, 2011; Serra-Millàs, 2016).

In respect to results on platelet [^3H]-PAR binding, similarly to what observed for BDNF, we cannot conclude that this is a specific biological correlate of reduced psychosocial skills in mild HD. Present SERT findings, albeit not decisive for our purposes, deserve awareness. As regards the role of 5-HT in HD, the data from the literature are apparently controversial. Indeed, low levels of 5-HT together with a reduced activity of tryptophan hydroxylase 2 (TPH₂) due the expression of *m*-Htt (Yohrling et al, 2002), were observed in a mouse model of the disease, whereas a region-dependent increase of 5-HT was detected in post-mortem brain of patients (Reynolds and Pearson, 1987). Beyond these findings, the lack of appreciable differences between platelet [^3H]-PAR binding parameters in stage-II HD subjects versus controls, as here obtained, would indicate that no appreciable 5-HT alteration take place in patients

showing mild HD symptoms, in the absence of a full-blown psychiatric, pain or metabolic disorder. Significant changes of platelet SERT, measured by [³H]-PAR, have been in fact observed in depression and anxiety (Iny et al, 1994), obsessive compulsive disorder (Marazziti et al, 1996), depression with suicidality (Verkes et al, 1997; Marazziti et al, 2001), fibromyalgia (Bazzichi et al, 2006) and obesity (Giannaccini et al, 2013).

It is also tempting to consider that platelet BDNF and SERT proteins can modulate each other under different conditions. For instance, a higher BDNF storage would enhance SERT expression in HD, since we observed a trend for a positive correlation ($r=0.40$) between platelet SERT B_{max} and BDNF amounts in patients, whereas an opposite trend between these two parameters, or a negative correlation ($r=-0.46$), was obtained in healthy controls, without reaching however, in both cases, the statistical significance (data not shown).

For what concerns instead the reported negative relationship between a low-affinity state of SERT protein and verbal anger perception in the groups of patients and controls, this seems rather an index of decreased emotion perception in the mature/elderly general population, regardless of the diagnosis of HD. In this regard, it should be mentioned that the affinity state of platelet SERT can vary with aging (Marazziti et al, 1998). On the other hand, our result further support the impact of 5-HT system on social behavior and emotion perception, including verbal anger recognition (Grossman et al, 2013; Boll and Gamer, 2014). It is also worth noting that, in a previous study, conducted in healthy subjects, younger than in present work, we reported a positive correlation between circulating oxytocin levels and [³H]-PAR binding K_D values (Marazziti et al, 2012), suggesting that this neuropeptide, directly involved in social behavior (Heinricks et al, 2009), can modulate SERT affinity. Anyway, other endogenous factors could affect SERT affinity states for 5-HT, linked to variations of the carrier's active conformation (Lau and Schloss, 2012). As for BDNF, deeper investigations are required: in this case, the study of the regulation of SERT protein phosphorylation states together the evaluation of 5-HT intra- and extra-platelet contents would provide useful information. Therefore, other molecular targets, such as neuroreceptors and signaling paths belonging to the 5-HTergic/catecholamine systems or to

neuropeptidergic modulation should be concomitantly considered to define more peculiar neurochemical features of impaired social behavior in HD.

Present study shows limitations linked to the small sample of subjects investigated, overall due to constraints in recruitment, as indicated in the Method section, as well as the high number of variables considered in respect of the sample size. However, to our knowledge, this is also the first examination that concomitantly measures BDNF and SERT in platelets of HD patients in relation to their clinical condition, psychosocial abilities and emotion recognition performance. We could not specifically relate BDNF levels and SERT binding parameters in platelets to social cognition impairment or HD features in our group of patients at the illness's stage II, except for BDNF and disease duration.

Anyway, the significant correlations obtained herein encourage pursuing the study, by possibly recruiting wider cohorts of subjects, by including drug-naïve patients, engaged at the time of diagnosis, or affected by more severe stages/phases of the disease. At the same time, present work indirectly suggests that the implementation of multidimensional and multidisciplinary approaches to identify behavioral and biochemical patterns of HD is a likely fruitful strategy to advance actual knowledge/management of this neurodegenerative disease and to possible delay terminal phases and improve patients' life quality.

Conflict of interest: None declared.

Figure legends

Figure 1. Intra-platelet BDNF amounts in controls and HD patients. BDNF levels were (mean \pm SEM): 2343 \pm 302 pg mg⁻¹ in controls (n=11) vs. 3023 \pm 212 pg mg⁻¹ in patients (n=13), resulting in a moderately greater BDNF content in platelets of HD patients than controls (two tailed *t*-test, .05 \leq P \leq .075).

Figure 2. [³H]-PAR binding parameters in platelet membranes of controls and HD patients: the expression (a) and affinity (b) of platelet SERT. (a): [³H]-PAR B_{max} was (mean \pm SEM): 1065 \pm 107 fmol mg⁻¹ in controls (n=11) vs. 916.3 \pm 70 fmol mg⁻¹ in patients (n=13); (b): [³H]-PAR K_D was: 0.14 \pm 0.03 nM in controls (n=11) vs. 0.18 \pm 0.025 nM in patients (n=13), (*t*-test, P>.05).

Figure 3. (a) Correlation between BDNF storage and HD duration in patients (n=13). Positive significant correlation, Pearson, P <.05. The dashed line represents the best fit from the linear regression analysis of data.(b) Correlation between platelet [³H]-PAR K_Ds and angry-face perception in HD patients (n=12). Negative significant correlation, Pearson, P<.05. The dashed line represents the best fit from the linear regression analysis of data.

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