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The platelet maximum number of A_{2A}-receptor binding sites (B_{max}) linearly correlates with age at onset and CAG repeat expansion in Huntington's disease patients with predominant chorea

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Abstract

Huntington's disease (HD) is caused by an expanded CAG mutation and may show a heterogeneous clinical presentation. To date, although the age at onset mostly depends on the expanded CAG repeat number, no validated easy-to-test biomarkers exist either for following up patients progression rate or for exactly predicting age at onset (defined as the time when motor clinical manifestations first became noticeable). We tested the function of A_{2A} receptor, strongly expressed in the brain striatum and peripheral cells, in patients' blood platelets and confirmed a maximum number of binding sites (B_{max}) higher than in controls (216 ± 9 versus 137 ± 7; $p = 0.0001$). We found a linear correlation between the receptor B_{max} and the expanded CAG repeat number ($n = 52$, $r^2 = 0.19$, $p = 0.0011$). When we selected the patients according to their clinical presentation (according to the predominating motor manifestations) and plotted the receptor B_{max} against patients' age at onset, we found a significant linear correlation only when considering those subjects with chorea predominant on all other motor symptoms ($n = 26$, $r^2 = 0.39$, $p = 0.0007$). Because the typical chorea may depend on early dysfunction of the striatum in HD, peripheral A_{2A} amplification in blood platelets might reflect a central dysfunction in this part of the brain. Further studies on a larger sample size should confirm whether the analysis of A_{2A}-receptor binding in patients' blood could be a useful clinical marker according to the patients' phenotype.

Keywords: Age at onset; Onset chorea; A_{2A}-receptor dysfunction; B_{max}; CAG repeat expansion; Platelets

Huntington's disease (HD) is caused by a CAG expansion mutation translated into an elongated poly(Q) stretch in huntingtin, a protein widely expressed in many tissues [14]. The initial motor symptoms may be heterogeneously manifested and characterized either by *chorea* or, in about 8% cases, by extrapyramidal symptoms other than choreic movements [10]. Severe behavioral changes may sometimes precede the movement disorder by many years [12,16]. Although the age at onset mostly depends on the expanded CAG repeat number, no changes in easy-to-test biological variables can exactly predict age at onset, nor its propensity to anticipate within families [15]. An aberrant amplification of adenosine A_{2A}-receptor signaling documented in peripheral blood cells of subjects with HD nevertheless implies that this cellular dysfunction may be related to clinical and genetic features [3].

We analyzed A_{2A}-receptor function in blood platelets from patients selected according to their clinical presentation. Because the dysfunction of the striatum, the brain structure most involved in HD, has been proposed as the main cause of chorea in HD [7], we analyzed receptor function focusing on patients in whom clinical features of chorea predominated over all other motor symptoms. We studied A_{2A} receptors in platelets because platelets are easily obtained in large amounts simply by drawing a blood sample, have an embryological origin common to neurons [1] and may resemble central neurons [4,5].

Patients were periodically evaluated by the Unified Huntington Disease Rating Scale (UHDRS) and genetic laboratory test-

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Table 1
Demographic and A_{2A}-receptor data

Subjects' category no. (men/women)	Mean age in years	Mean CAG expanded repeats (range)	Mean age at onset in years (range)	Mean Huntington disease duration in years (range)	Mean loss of TFC units per years (range)	Mean loss of DS units per years (range)	Mean chorea score (range)	Mean chorea + dystonia scores (range)	B_{max} (fmol/mg protein) (range)
Patients									
With predominant chorea 26 (11/15)	55.0 ± 2.2	44.77 ± 0.63 (41–54)	43.23 ± 2.04 (27–66)	11.81 ± 1.02 (5–26)	0.75 ± 0.06 (0.16–1.3)	3.65 ± 0.34 (1.15–7.0)	16.1 ± 1.3 (6–28)	25.9 ± 2.20 (8–40)	222 ± 11 (88–319)
With atypical symptoms other than chorea 19 (12/7)	43.5 ± 2.8	47.68 ± 1.84 (39–70)	34.16 ± 3.22 (12–63)	13.42 ± 1.06 (5–21)	0.8 ± 0.09 (0.25–1.6)	4.05 ± 0.65 (0.93–9.72)	8.9 ± 1.6 (0–19)	18.9 ± 2.43 (0–34)	207 ± 15 (112–393)
Whole cohort 45 (23/22)	51.5 ± 1.9	46 ± 0.88 (39–70)	39.40 ± 1.89 (12–66)	12.49 ± 0.74 (5–26)	0.77 ± 0.05 (0.16–1.6)	3.82 ± 0.33 (0.93–9.72)	12.97 ± 1.1 (0–28)	22.85 ± 1.73 (0–40)	216 ± 9 (88–393)
Unaffected subjects									
Asymptomatic mutation carriers 7 (4/3)	35.3 ± 1.5	43.43 ± 0.65 (41–46)	–	–	–	–	–	–	194 ± 19 (118–238)
Controls 42 (20/22)	47.2 ± 1.1		–	–	–	–	–	–	137 ± 7 (42–284)

Values are given as mean ± S.E. TFC: total functional capacity; DS: disability score. No statistical differences in Bmax were found among the HD subjects' categories (including the asymptomatic mutation carriers).

ing performed in accordance with published protocols including informed consents for patients with symptomatic and asymptomatic HD [9,13]. All clinical and genetic data including age at onset (defined as the time when motor clinical manifestations - e.g. extrapyramidal symptoms - first became noticeable) [10], onset symptoms, disease duration and symptom progression rate (loss of units per year of the Total Functional Capacity (TFC) Scale and Disability Scale (DS) scores) [13], are stored in a data bank [11]. We obtained a 50 ml blood sample, after informed consent, from a total of 52 HD subjects (45 symptomatic and seven unaffected mutation carriers; 27 men/25 women) and 42 age-matched healthy controls (20 men/22 women). From the whole cohort, 26 patients (11 men/15 women), had age at onset and a clinical course characterized by choreic movements that clearly predominated over other neurological symptoms; 19 (12 men/7 women) had atypical motor manifestations other than chorea since the onset, although they may have manifested slight features of chorea years after the onset, during HD symptom progression [10,12]. Clinical HD variants were selected according to a previously published method that took into account the single motor score items in the UHDRS [2,8,10]. In each subject with chorea-predominant HD, the total chorea score divided by the dystonia score was greater than 1 [2]. Among the patients who manifested chorea over time predominating over other symptoms, we selected those subjects with a disease history of at least 5 years. Demographic and clinical details of all patients are reported in the Table 1. We excluded from the analysis those subjects with cardiovascular or psychiatric features [17]. Most patients were taking small-to-moderate doses of atypical neuroleptics (risperidone 0.5–2 mg and olanzapine 5–10 mg) known to leave receptor function unchanged [17].

Blood platelets were isolated within 6–8 h after samples were drawn. Membranes from human platelets were prepared as previously described [18] and used for radioligand binding assays. For saturation binding experiments membranes obtained from platelets were incubated with 8–10 concentrations of the A_{2A} antagonist ([^{3}H]-4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]-ethyl)phenol) [^{3}H]-ZM 241385 in the range 0.01–10 nM at 4 °C for 60 min [17] (on-line Supplementary Fig. I). Non-specific binding was determined in the presence of 10 μM *N*-ethylcarboxamido-adenosine (NECA). Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/C glass fiber filters with a Brandel cell harvester. The filter bound radioactivity was counted using a Beckman liquid scintillation counter.

For statistical analysis we used a nonparametric test (Mann–Whitney *U*-test) to compare B_{max} values in patients with HD and healthy controls and between each group of HD subjects. A simple regression model was used to analyze the linear dependence of clinical variables (e.g. age at onset, disease duration, symptom progression rate) on the maximum number of binding sites (B_{max}). All values are expressed as mean ± S.E. Significance was considered at $p < 0.05$.

The maximum number of binding sites for the A_{2A} receptor was larger in symptomatic and asymptomatic HD subjects than in controls (216 and 194 versus 137, respectively, $p = 0.0001$).