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**Characterization of adenosine A₂ receptors of peripheral blood
mononuclear cells of patients with fibromuscular dysplasia**

François SILHOL^{1*}, Marion MARLINGE^{2,3*}, Claire GUIOL^{2*}, Mohamed CHEFROUR³,
Patrick MACE³, Christine CRIADO², Nathalie KIPSON², Bernard VAISSE¹, Donato
VAIRO², Gabrielle SARLON¹, Jean RUF², Anne DONNET⁴, Laurie BRUZZESE²,
Giovanna MOTTOLA², Régis GUIEU^{2,3,4*}, Emmanuel FENOUILLET^{2,5*}

1 Hypertension Department, Pôle Cardio-Vasculaire, Hôpital de la Timone

264 Avenue Saint-Pierre 13385 Marseille cedex 05

2 Aix Marseille Université, INSERM, INRA, C2VN

3 Laboratory of Biochemistry, Hôpital de la Timone

4 Chronic Pain and Headache Centre, Hôpital de la Timone

5 Institut des Sciences Biologiques, CNRS, 75016 Paris, France

* These authors contribute equally to the work

Correspondence to: François Silhol, Hypertension Department, Pôle Cardio-Vasculaire,
Hôpital de la Timone, 264 Avenue Saint-Pierre, 13385 Marseille cedex 05. E-mail:
francois.silhol@ap-hm.fr

Fibromuscular dysplasia (FMD) is a non-atherosclerotic, non-inflammatory vascular disease characterized by unexplained systemic hypertension under the age of 35, migraine-like headache, epigastric or cervical bruit, arterial stenosis, aneurysm rupture, intracranial/renal infarction, renal damage and stroke^{1,2}.

Although the gold standard for the diagnosis remains catheter angiography, biological markers would be helpful as the delay from first symptom to diagnosis is long. It is of note that most patients are females¹, which is in agreement with high expression of progesterone receptors in renovascular FMD³.

Adenosine, an ATP derivative, may be implicated in FMD pathophysiology. Adenosine exerts its effects via its membrane receptors: A₁R, A_{2A}R, A_{2B}R, and A₃R⁴. While A₁R and A₃R control heart rhythm and the adaptive response to ischemia-reperfusion, respectively, A_{2A}R and A_{2B}R impact blood flow⁴. Although acute release of adenosine improves vascular blood flow, and hence is cardiovascular protective⁴, chronic exposure to high adenosine plasma level (APL) has deleterious effects especially via A_{2B}R activation that promotes vascular fibrosis^{5,6}.

We hypothesized therefore that changes in APL and expression of A_{2A}R and A_{2B}R are associated with FMD.

Patients

Adult patients with multifocal FMD (diagnosed using CT scan or duplex ultrasound) were included according to current criteria⁷. Common migraine diagnosis was in accordance with the ICHD-3 classification. Patients with essential hypertension (EH) were also included. Control group, matched for age and sex, was recruited among the medical staff.

APL measurement

Blood samples were collected and processed using tubes containing stop solution and APL was measured by liquid chromatography-tandem mass spectrometry⁸ (Shimadzu UFLC XR, Marne-la-Vallée, F).

A₂ receptors production by peripheral blood mononuclear cells (PBMCs)

PBMCs were chosen because the pharmacological profile of adenosine receptors expressed at their surface mirrors that of their counterparts associated with cardiovascular tissues⁸. PBMCs were isolated from blood using the Vacutainer-CPT system (Becton-Dickinson, Franklin-Lakes, NJ). A_{2A}R production was assessed by Western blot and results were expressed in arbitrary units (AU) according

to a procedure previously validated⁸. A₂BR production was similarly addressed except the use of a commercial antibody (Abcam®, Cambridge, UK).

Statistical analysis

Quantitative variables are reported as mean±standard deviation (SD) or median and interquartile range (IQR), and qualitative variables as numbers and percentages. A variance analysis (ANOVA two ways) was used for intergroup comparisons. Two-sided tests were performed and P-value <0.05 was considered as significant.

Patients

Clinical characteristics are given (Table 1). Among the 24 FMD patients, 14 (58%) had headaches and 6 (25%) typical migraine crisis ("FMD with migraine" patients thereafter). Twenty-one FMD patients (87%) were treated for hypertension (SBP: mean[range]: 128[115-140]mmHg; DBP:78[63-90]mmHg) at time of blood collection. The EH patients were also treated for hypertension (SBP: 133[118-149] mmHg; DBP:70[54-85] mmHg).

APL

APL was high in FMD patients (Fig.1, panel A; FMD vs controls: 2.68±0.95 vs 0.59±0.13 μM; p<0.01) but was not affected by the presence of associated migraine (FMD vs FMD with migraine: 2.68±0.95 vs 2.70±1.30 μM; p=0.7). APL was higher in FMD with migraine vs migraine patients (2.70±1.30 vs 0.56±0.14 μM; p<0.001). APL was similar in migraine patients and controls (0.56±0.14 vs 0.59±0.13 μM; p>0.05) and higher in EH patients vs controls (0.82±0.15 vs 0.59±0.13 μM; p = 0.0015).

Receptor Production

A_{2A}R production was affected neither by FMD (Fig.1, panels B & C; FMD vs controls: 1.23±0.15 vs 1.19±0.08 AU; p=0.5) nor by the presence of associated migraine (FMD vs FMD with migraine: 1.23±0.15 vs 1.27±0.12 AU; p = 0.5). A_{2A}R production was also affected neither by migraine nor by hypertension (migraine: 1.16±0.12 AU; EH: 1.27±0.18 AU; controls: 1.19±0.08 AU; p= 0.29 and 0.86, respectively).

A_{2B}R expression was high in FMD patients (Fig.1, panel B & C; FMD vs controls: 2.46±0.77 vs 1.20±0.12 AU; p<0.001) but was not affected by the presence of associated migraine (FMD vs FMD with migraine: 2.46±0.77 vs 2.58±0.97 AU; p=0.9). A_{2B}R production was higher in FMD with migraine vs migraine patients (FMD with migraine vs migraine: 2.58±0.97 vs 1.32±0.25 AU;

$p < 0.001$). Finally $A_{2B}R$ production did not differ in migraine patients or EH patients vs controls (migraine: 1.32 ± 0.25 AU; EH: 1.27 ± 0.16 AU; controls: 1.20 ± 0.12 AU; $p = 0.2$ and 0.32 , respectively).

We show here that FMD patients have high APL and high $A_{2B}R$ production, irrespective of the presence of migraine.

Various reports are consistent with a role of $A_{2B}R$ in the maintenance/progression of FMD: although upregulation of $A_{2B}R$ protects against acute vascular injury via TNF-alpha in an animal model⁹, activation of $A_{2B}R$ leads to vascular smooth cell apoptosis⁶ and fibrosis⁵. This supports a role of $A_{2B}R$ activation in the maintenance/progression of FMD. Interestingly, $A_{2B}R$ has low affinity for adenosine⁴ and is activated only in case of high APL that are in the range of the levels found here in FMD patients.

We found no significant difference in $A_{2A}R$ expression in FMD patients as well as in migraine patients compared with controls. $A_{2A}R$ is highly expressed in patients with essential hypertension that normalized after treatment using doxazosin but not using propranolol¹⁰. Here, despite a context of hypertension, no significant difference in $A_{2A}R$ expression was found between FMD with associated hypertension and controls, probably because of the normotensive status of patients at the sampling time.

Whether FMD is a rare or common disease is subject of debate, probably because of the difficulty of diagnosis and the time delay from onset to diagnosis. Indeed, the mean age for first symptom is around 47 years while the mean age at diagnosis is 52 years¹. FMD may affect every arterial bed but it mostly affects the renal and extracranial carotid as well as vertebral arteries¹. Thus, FMD is rather a systemic disease. It is therefore not surprising that FMD is associated with systemic adenosinergic abnormalities as observed here using PBMCs.

Our results indicate that FMD is associated with high $A_{2B}R$ expression and high APL, irrespective of the presence of migraine-like headache. The mechanistic role of $A_{2B}R$ in FMD needs study.

Measurement of $A_{2B}R$ production by using a simple blood collection may represent a valuable biological marker of FMD and a help in FMD diagnosis.

Key words: fibromuscular dysplasia, migraine, adenosine A_2 receptors, adenosine

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Conflict of interest: None

References:

1. Olin JW, Froehlich J, Gu X, Bacharach JM, Eagle K, Gray BH et al. The united states registry for fibromuscular dysplasia: results in the first 447 patients. *Circulation* 2012; **125**:3182-90.
2. van Twist DJL, de Leeuw PW, Kroon AA. Renal artery fibromuscular dysplasia and its effect on the kidney. *Hypertens Res*. 2018; **41**:639-48.
3. Silhol F, Sarlon-Bartoli G, Daniel L, Bartoli JM, Cohen S, Lepidi H et al. Intranuclear expression of progesterone receptors in smooth muscle cells of renovascular fibromuscular dysplasia: a pilot study. *Ann Vasc Surg* 2015; **29**:830-5.
4. Burnstock G. Purinergic signaling in the cardiovascular system. *Circ Res* 2017; **120**:207-28.
5. Borea PA, Gessi S, Merighi S, Vincenzi F, Varani K. Pharmacology of adenosine receptors: The State of the Art. *Physiol Rev* 2018; **98**:1591-1625.
6. Peyot ML, Gadeau AP, Dandré F, Belloc I, Dupuch F, Desgranges C. Extracellular adenosine induces apoptosis of human arterial smooth muscle cells via A(2b)-purinoceptor. *Circ Res* 2000; **86**:76-85.
7. Slovut DP, Olin JW. Fibromuscular dysplasia. *N Eng J Med* 2004; **350**: 1862-71.
8. Gariboldi V, Vairo D, Guieu R, Marlingue M, Ravis E, Lagier D et al. Expressions of adenosine A2A receptors in coronary arteries and peripheral blood mononuclear cells are correlated in coronary artery disease patients. *Int J Cardiol* 2017; **230**:427-31.
9. Yang D, Koupenova M, McCrann DJ, Kopeikina KJ, Kagan HM, Schreiber BM, Ravid K. The A2b adenosine receptor protects against vascular injury. *Proc Natl Acad Sci U S A* 2008; **105**:792-6.
10. Varani K, Manfredini R, Iannotta V, Pancaldi C, Cattabriga E, Uluoglu C et al. Effects of doxazosin and propranolol on A2A adenosine receptors in essential hypertension. *Hypertension* 2002; **40**:909-13.

Figure 1 legend:

Adenosine plasma level (Panel A) and A_{2A}- or A_{2B}-receptor expression (Panel B) in patients. a: Whole FMD patient population; b : FMD without migraine-like headache; c: migraine patients; d: FMD with migraine-like headache; e: hypertensive patients (hypertension); f: controls. Examples of Western blot of A_{2A}- (Top) and A_{2B}- (Bottom) receptor production by peripheral blood mononuclear cells.* p<0.01 compared with controls. MW: molecular weight.

