



## **Correlation between low adenosine A2A receptor expression and hypercholesterolemia: A new component of the cardiovascular risk?**

Donato Vairo, Carola Giacobbe, Claire Guiol, Marie-Charlotte Chaptal, Maria Donata Di Taranto, Laurie Bruzzese, Jean Ruf, Régis Guieu, Giuliana Fortunato, Emmanuel Fenouillet, et al.

### **► To cite this version:**

Donato Vairo, Carola Giacobbe, Claire Guiol, Marie-Charlotte Chaptal, Maria Donata Di Taranto, et al.. Correlation between low adenosine A2A receptor expression and hypercholesterolemia: A new component of the cardiovascular risk?. *Biochimica et Biophysica Acta Molecular and Cell Biology of Lipids*, 2020, 1866, 10.1016/j.bbalip.2020.158850 . hal-03798635

**HAL Id: hal-03798635**

**<https://hal.science/hal-03798635>**

Submitted on 6 Oct 2022

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Manuscript (No.: BBALIP-20-300) entitled “Correlation Between Low Adenosine A<sub>2A</sub> Receptor Expression and Hypercholesterolemia: a New Component of the Cardiovascular Risk?”.

#### Highlights (mandatory)

3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

- Familial Hypercholesterolemia is associated to low Adenosine A<sub>2A</sub> Receptor expression.
- Expression of A<sub>2A</sub>R and plasma cholesterol levels are negatively correlated in FH.
- Plasma cholesterol levels and A<sub>2A</sub>R expression in PBMCs are correlated in humans.

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125 All authors have nothing to disclose.

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## **Correlation between Low Adenosine A<sub>2A</sub> Receptor Expression and Hypercholesterolemia: a New Component of the Cardiovascular Risk?**

The adenosine A<sub>2A</sub> receptor (A<sub>2A</sub>R) plays a major role in the physiology of the cardiovascular and immune systems. Its activation induces vasodilation and immunosuppression, two adaptive responses to ischemia and inflammation [1]. Evidences from cell cultures or animal models - but not human studies - indicate that as a surface protein embedded in the cholesterol-rich plasma membrane, A<sub>2A</sub>R is functionally dependent on membrane cholesterol composition that, in turn, depends on plasma cholesterol level [2]. Thus, it can be hypothesized that abnormal A<sub>2A</sub>R expression develops in humans in a context of hypercholesterolemia. Additional clues are that elevated cholesterol level is a major risk factor for atherosclerotic cardiovascular diseases as it facilitates endothelial dysfunction and chronic inflammation to promote atherosclerosis, while low systemic A<sub>2A</sub>R expression is often found in cardiovascular diseases [3, 4].

In this initial investigation, we examined whether a relationship exists in humans between A<sub>2A</sub>R expression and plasma cholesterol: i) A<sub>2A</sub>R expression was explored in vivo using peripheral blood mononuclear cells (PBMCs) as properties of A<sub>2A</sub>R produced by PBMCs reflect those of A<sub>2A</sub>R produced by cardiovascular tissues including aortic, coronary and artery tissues [3]. PBMCs were isolated (CPT™ Cell Preparation system; Becton-Dickinson) and A<sub>2A</sub>R expression was assessed using Western blot and densitometry analysis [3]; ii) to have elevated plasma cholesterol, we took advantage of the context of Familial Hypercholesterolemia (FH), a severe genetic hyperlipidemia characterized by elevated blood levels of total-cholesterol (T-C) and low-density lipoprotein-cholesterol (LDL-C) [5]. We recruited 10 untreated FH Caucasian patients (Mean age (SD): 28.1 (18.2) years; 40% women) with heterozygous mutations causing a 45-65% residual activity of the LDL receptor

and elevated total-cholesterol (T-C) and low-density lipoprotein-cholesterol (LDL-C) blood values (T-C: median [range]: 346 [323-423] mg/dL; LDL-C: 269 [254-336] mg/dL) [5]. We also recruited as controls 10 healthy Caucasian subjects (Mean age (SD): 22.6 (0.7) years; 50% women; no cardiovascular risk; T-C: 168 [146-191] mg/dL; LDL-C: 101 [69-126] mg/dL). An informed consent for the study was obtained for experimentation with human subjects. The study was approved by the Ethical Committee of the Università degli Studi di Napoli Federico II (Number 157/13, September 9, 2013).

We found reduced A<sub>2A</sub>R expression in FH patients vs healthy subjects (Mean value (SD): 54.0 (22.46) vs 125 (32.23) fmol/mg protein; figure 1A). Despite the small sample size, such difference was highly significant ( $p < 0.0001$ , a  $p$  value  $< 0.05$  being considered to be significant). Additionally, we observed in FH patients a negative correlation between A<sub>2A</sub>R expression and plasma cholesterol (T-C: Pearson  $r = -0.8517$ ;  $r^2 = 0.7254$ ;  $p = 0.0018$ ; LDL-C:  $r = -0.7556$ ;  $r^2 = 0.5709$ ;  $p = 0.0115$ ; figure 1B).

Two distinct mechanisms can potentially explain the decrease of A<sub>2A</sub>R expression observed in FH patients. On the one hand, it may constitute an adaptive response to the atherosclerotic process generated by hypercholesterolemia for the reason that A<sub>2A</sub>R knockout in bone-marrow-derived cells of a murine atherosclerosis model induces a pro-inflammatory phenotype together with the development of smaller atherosclerotic lesions due to apoptosis [6]. Accordingly, the decrease of A<sub>2A</sub>R expression may be induced to protect FH patients against atherosclerosis. On the other hand, if the reduced A<sub>2A</sub>R expression in PBMCs of FH patients reflects reduced A<sub>2A</sub>R expression in endothelial cells as A<sub>2A</sub>R production is submitted to systemic regulation [3], then a decreased expression probably compromises both the inflammatory and the vasodilatory responses to ischemic disorders with deleterious effects in FH. Previous reports support this latter view: i) while effects of hypercholesterolemia on A<sub>2A</sub>R in endothelial cells remain to be examined, adenosine weakly dilates coronary vasculature in dyslipidemic patients, suggesting an alteration of A<sub>2A</sub>R vasodilatory capacity

[7]; ii) A<sub>2A</sub>R-activation in macrophage cultures inhibits the atherogenic process of foam cell formation [8]; iii) high-resolution crystal structure analysis highlights cholesterol-binding sites in A<sub>2A</sub>R, which supports a direct functional regulation of A<sub>2A</sub>R by cholesterol [9].

In both cases, the correlation between plasma cholesterol and A<sub>2A</sub>R expression we found here in humans may constitute a new facet of the influence of cholesterol on the cardiovascular risk.

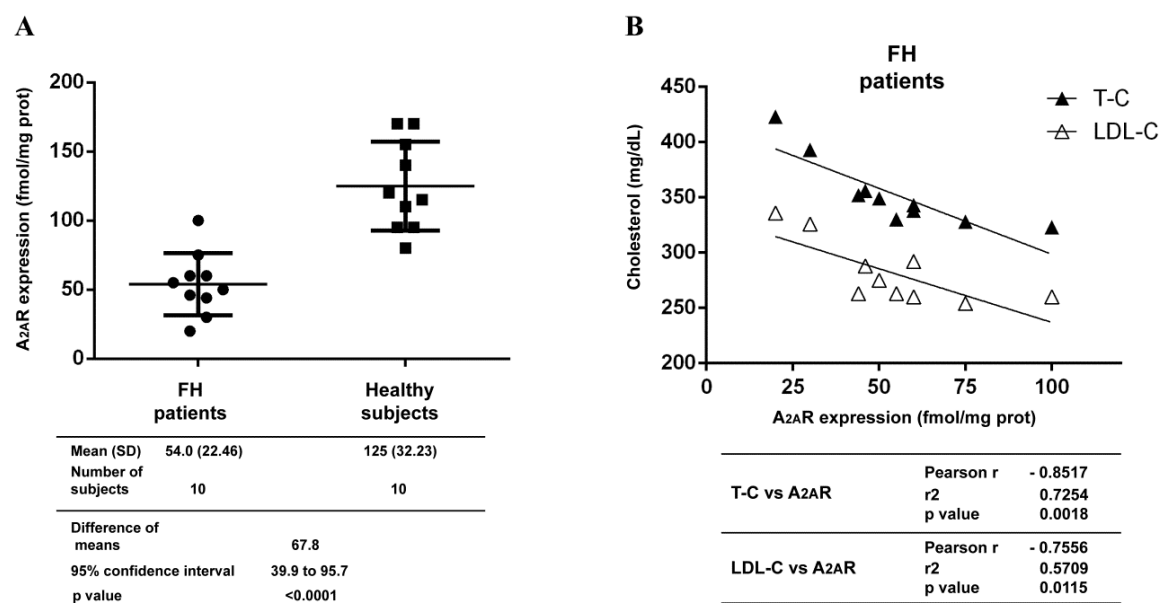
### *Acknowledgements*

The authors would like to thank S. Bonatti (Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy) for critical reading of the manuscript.

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**Figure 1**

$A_{2A}R$  expression by PBMCs is reduced in FH patients vs healthy subjects (A) and negatively correlated to blood levels of total-cholesterol (T-C) or low-density lipoprotein-cholesterol (LDL-C) (B). After Western blotting and densitometry analysis,  $A_{2A}R$  expression was quantified (fmol/mg protein) by reference to a standard curve obtained with known amounts of PBMC-associated  $A_{2A}R$ . Values were expressed as Mean (SD) of three separate experiments. The unpaired t test comparison with Welch's correction was used for intergroup comparisons. Pearson's r coefficient of correlation and the coefficient of determination  $r^2$  were used for the correlation study; p value < 0.05 was considered to be significant.