

Letter to the Editor

Cytosolic phospholipase A₂ group IVA is over-expressed in patients with persistent asthma and regulated by the promoter microsatellites*To the Editor:*

Severe asthma is a complex disease influenced by genetic and environmental factors.¹ Eighty-five-kilodalton cytosolic phospholipase A₂ group IVA, also known as cytosolic phospholipase A₂ α (cPLA₂ α), is a major intracellular form of phospholipases A₂.² It is a rate-limiting enzyme in arachidonic acid liberation from cellular membranes and further eicosanoids production.² Recent studies have shown an importance of cPLA₂ α in overall eicosanoid production in human beings.³ In addition, cPLA₂ α knockout mice studies have provided critical evidence of its involvement in asthma and other chronic inflammatory diseases.⁴ We have previously shown that microsatellite fragments (T)_n and (CA)_n in the promoter region of cPLA₂ α gene (*PLA2G4A*), although highly polymorphic, are significantly shorter in patients with severe asthma than in healthy subjects.⁵ Moreover, we have noticed that the shorter alleles of these sequences caused an increase in *PLA2G4A* *de novo* transcription.⁵ Therefore, the aim of the current study was to evaluate the influence of these microsatellite *PLA2G4A* promoter fragments on cPLA₂ α mRNA and protein expression and subsequent eicosanoids generation together with the overall comparison of these parameters between patients with asthma and healthy controls.

Sixteen patients with severe asthma were enrolled to the first part of the study on the basis of the (T)_n and (CA)_n microsatellites length in the *PLA2G4A* gene promoter. The second part of the study involved 8 patients with persistent severe asthma and 5 patients with persistent nonsevere asthma randomly selected from asthmatic group treated in the Outpatient Unit of the Department of Immunology, Rheumatology and Allergy in Lodz, Poland. The sex-matched healthy individuals group consisted of 8 unrelated, nonatopic subjects with a negative family history of allergy and asthma. Asthma was defined according to Global Initiative for Asthma 2008 criteria.⁶ The severity of the disease was assessed according to the American Thoracic Society Workshop on Refractory Asthma 2000 report.⁷ The study was approved by the appropriate ethics committee, and informed consent was obtained from every subject before the study. Genotyping was performed by direct sequencing with specific primers. Gene expression was evaluated by real-time PCR, compared with the *GAPDH* gene and shown as relative quantity (RQ). cPLA₂ α protein expression was evaluated by immunoblotting with β -actin as a positive standard and shown in OD units. 15-Hydroxyeicosatetraenoic acid, leukotriene C₄, and prostaglandin E₂ were measured using a competitive ELISA method in the supernatants of PBMCs cultured with or without calcium ionophore and shown in picograms per milliliter. Comparisons were performed by using Mann-Whitney *U* tests. Data shown are means $\pm$ SEMs. Values of *P* < .05 were considered statistically significant.

We analyzed the influence of (T)_n and (CA)_n microsatellites on the cPLA₂ α mRNA, protein expression, and downstream eicosanoids generation. Eight patients with severe asthma with shorter alleles (CA)₁₂₋₁₈ and (T)₁₇₋₃₈ and 8 patients with longer alleles (CA)₁₉₋₂₄ and (T)₃₉₋₄₆ of analyzed microsatellites regions in the

PLA2G4A promoter were enrolled in this study. Classification into each length group was done on the basis of the data from our previous studies.⁵ In the current study, we found significantly higher expression of cPLA₂ α mRNA (RQ = 5.72 ± 1.39) in PBMCs from patients with shorter alleles (CA)₁₂₋₁₈ and (T)₁₇₋₃₈ than in patients with longer promoter microsatellites variants (RQ = 2.36 ± 0.34 ; *P* = .002; Fig 1, A). Furthermore, in patients with severe asthma carrying shorter alleles of analyzed promoter microsatellites, the cPLA₂ α protein expression was also significantly increased (OD = 27.03 ± 3.35) compared with patients with severe asthma but carrying longer (CA)₁₉₋₂₄ and (T)₃₉₋₄₆ alleles (OD = 8.83 ± 1.98 ; *P* = .004; Fig 1, B; Fig 2, A). Moreover, cPLA₂ α overexpression was followed by significantly higher basal expression of 15-hydroxyeicosatetraenoic acid (*P* = .002) and prostaglandin E₂ (*P* = .011) but not leukotriene C₄ in carriers of shorter alleles (Fig 1, C and D).

To evaluate the overall cPLA₂ α mRNA and protein expression in patients with asthma regardless of the *PLA2G4A* promoter structure, we randomly selected patients with severe and nonsevere asthma and healthy subjects. We found that the cPLA₂ α mRNA expression in PBMCs from patients with severe asthma (RQ = 5.95 ± 1.14) did not differ from nonsevere asthma (RQ = 3.39 ± 0.86 ; *P* > .05) but was significantly higher than in PBMCs from healthy subjects (RQ = 1.71 ± 0.34 ; *P* = .021; Fig 1, E). To assess the final cPLA₂ α protein expression in the same groups of subjects, we performed immunoblotting, finding confirming results. The cPLA₂ α protein expression in PBMCs from patients with severe asthma (OD = 18.81 ± 3.05) was similar to nonsevere asthma (OD = 11.45 ± 4.95) but significantly higher than in healthy controls (OD = 3.77 ± 1.48 ; *P* = .0021; Fig 1, F; Fig 2, B). However, the basal and stimulated expression of eicosanoids was similar in each of the analyzed groups.

Here we showed that the length of microsatellite sequences (T)_n and (CA)_n in the promoter region of *PLA2G4A* gene have a direct impact on the cPLA₂ α mRNA and final protein expression in patients with severe asthma. Longer microsatellites sequences caused lower cPLA₂ α mRNA and protein expression. In the previous study, we demonstrated that (T)_n and (CA)_n microsatellites serve as potent inhibitory elements of *PLA2G4A* *de novo* transcription and that the inhibitory potential of these regions decreases with their length.⁵ Moreover, we showed previously that the group of shorter alleles of the (CA)_n microsatellite region (*n* = 12-18) and the group of shorter alleles of (T)_n repeats region (*n* = 17-38) occurred significantly more often in patients with severe asthma compared with healthy controls.⁵ Therefore, our current results might be considered as evaluation data on functional effects of analyzed (T)_n and (CA)_n microsatellites because we demonstrated here additional lines of evidence on mRNA and protein level, as well as an effect on eicosanoids generation. The inhibitory role of (T)_n and (CA)_n microsatellites, demonstrated in detail in the previous⁵ and current study, is consistent with the data on *PLA2G4A* promoter organization and transcriptional regulation obtained by Wu et al⁸ and Dolan-O'Keefe et al.⁹ Also, other genes have been demonstrated to be regulated by microsatellites in their promoter regions, which have determined functional effects. Uhlemann et al¹⁰ proposed a role for variation in the promoter TA dinucleotide microsatellite

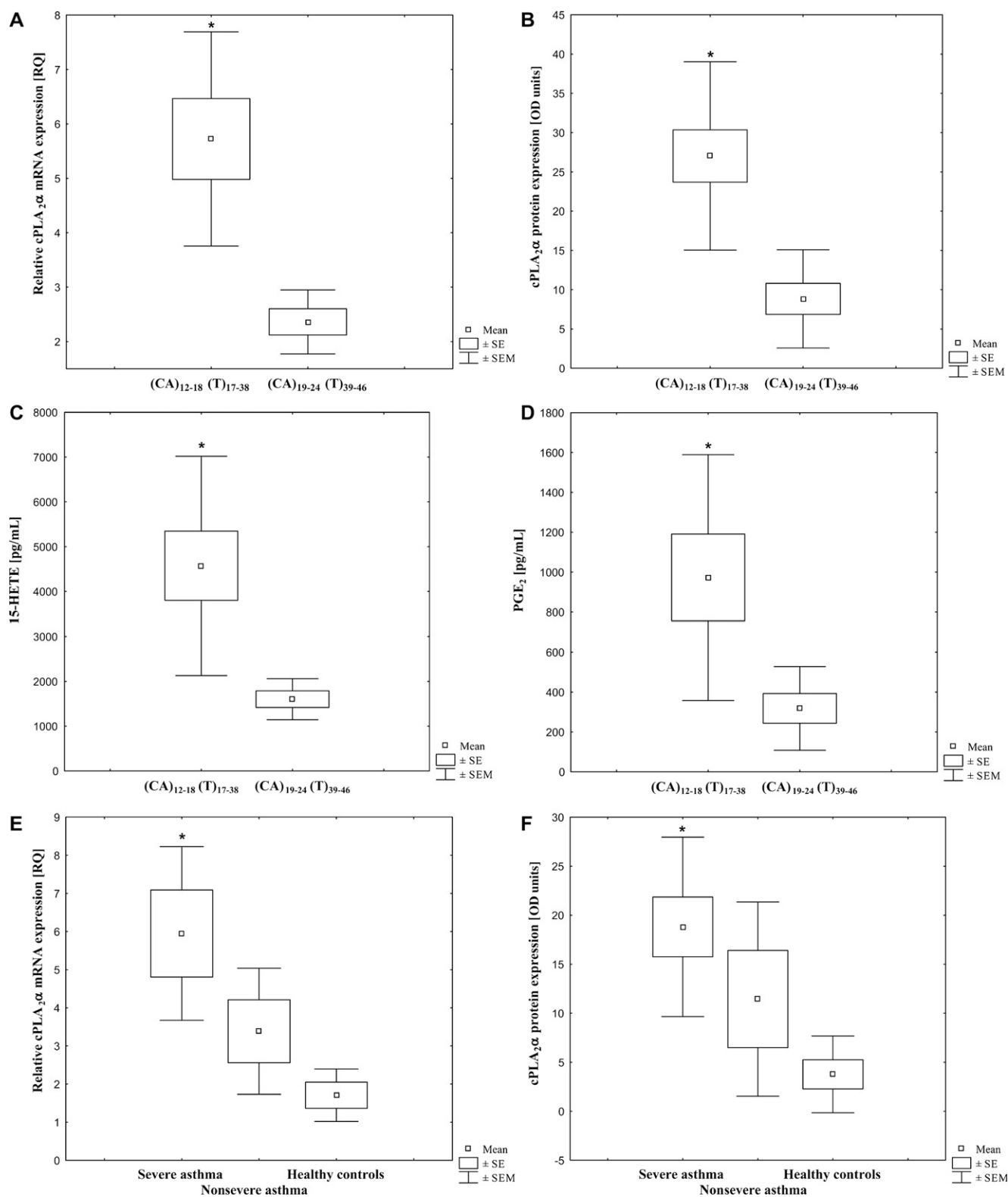


FIG 1. A-D, cPLA₂α mRNA, cPLA₂α protein expression, and eicosanoids generation in PBMCs from patients with severe asthma carrying shorter (CA)₁₂₋₁₈(T)₁₇₋₃₈ or longer (CA)₁₉₋₂₄(T)₃₉₋₄₆ alleles of analyzed *PLA2G4A* promoter microsatellites. A, cPLA₂α mRNA expression. Data are expressed as means ± SEMs of RQ compared with control gene expression. **P* < .05. B, cPLA₂α protein expression. Data are expressed as means ± SEMs of optical density units. **P* < .05. C, 15-Hydroxyeicosatetraenoic acid (15-HETE) and prostaglandin E₂ (PGE₂) generation (D). Data are shown in mean concentrations ± SEMs. **P* < .05. cPLA₂α mRNA (E) and cPLA₂α protein expression (F) in PBMCs of patients with severe and nonsevere asthma and healthy controls. **P* < .05 compared with healthy controls.

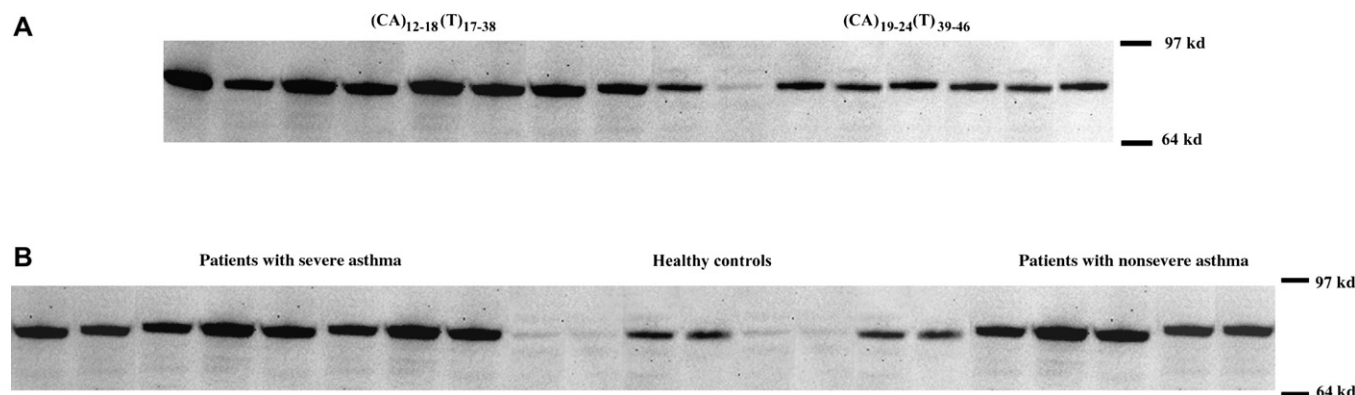


FIG 2. Images of representative cPLA₂α immunoblotting experiments in patients with severe asthma carrying shorter (CA)₁₂₋₁₈(T)₁₇₋₃₈ or longer (CA)₁₉₋₂₄(T)₃₉₋₄₆ alleles of analyzed *PLA2G4A* promoter microsatellites (A) and in patients with severe asthma and nonsevere asthma and healthy controls (B).

on the levels of gp91phox subunit of nicotinamide adenine dinucleotide phosphate oxidase gene (*CYBB*) transcription through a DNA looping mechanism, which further correlates with reactive oxygen species generation and malaria severity. Recently it has been shown that the mechanism of hypoxia-inducible factor-1 (HIF-1) transcription factor joining Z-DNA structure of (GT/AC)_n microsatellite in the promoter of *SLC11A1* gene might have an impact on intracellular bacteria infection and leishmaniasis.¹¹

Additional observations strengthening our initial hypothesis of cPLA₂α contribution to asthma pathogenesis are data on comparative analysis of cPLA₂α mRNA and protein expression in patients and healthy subjects randomly selected regardless of genotypes. Although we found similar expression of cPLA₂α in patients with severe and nonsevere asthma, it was still strongly overexpressed compared with healthy controls. Obviously, the overall high prevalence of shorter alleles of studied microsatellites in patients with severe asthma, which was shown in our previous study,⁵ might influence such results. On the other hand, the selection was performed at random according to drawing of lots from all patients with asthma treated in our unit. Although high expression of cPLA₂α in patients with asthma did not increase generation of eicosanoids by PBMCs, it might act locally in the bronchi,¹² delivering arachidonic acid for enzymes of eicosanoids pathway at the direct site of inflammation.¹³ It could also influence other proinflammatory gene expression.¹⁴

In summary, our data suggest that (CA)_n and (T)_n microsatellites in the *PLA2G4A* promoter determine cPLA₂α *in vivo* expression in patients with severe asthma and the overexpression of cPLA₂α in persistent asthma might play a role in its pathogenesis.

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