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PROGNOSTIC VALUE OF S100 PROTEINS IN FAMILIAL MEDITERRANEAN FEVER

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MEFV (Mediterranean Fever) gene is responsible for Familial Mediterranean Fever (FMF). Association of the MEFV gene mutations with clinical conditions other than FMF has recently been a subject of discussion where patients with other inflammatory conditions have also been diagnosed with FMF.

***Keywords*:** FMF, S100A4, inflammation, sandwich-ELISA.

Introduction. Familial Mediterranean Fever (FMF) is a severe systemic autoinflammatory disorder characterized by recurrent onsets of febrile attacks affecting mainly Big Middle East population with the increasing incidence among western European population due to the incremental rate of migration [1]. In this study we present clinical data from 20 000 FMF patients and discuss the clinical manifestation and genotype penetrance of individuals also affected with other disorders [2]. FMF is caused by recessively inherited mutations in MEFV, which encodes pyrin [3, 4]. Mutated pyrin is associated with the loss of delicate control of the inflammatory pathways, which results in a prolonged or augmented systemic inflammation that predisposes these patients and carriers of the MEFV mutation to a pro-inflammatory state. This increased inflammation might lead to susceptibility to various inflammations associated comorbidities in FMF patients and even in carriers [5]. Among them are such devastating complications of FMF as amyloidosis in various organs including heart, various vasculitides and the emerging problem of atherosclerosis as well as coexistence with other rheumatological autoimmune disorders. Moreover, these facts allow us categorizing FMF, which is authentically serious inflammatory disorder also as a high risk factor for other devastating conditions. That is why studies on key molecular factors and pathways involved in the pathogenesis of FMF and its progression into the life-threatening conditions (amyloidosis, cardiovasculitis and others) is a vital issue in several endemic regions and increasingly important for western countries [4].

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Members of the S100 family proteins (S100A7, S100A8/A9 and S100A12) have been associated with various types of severe chronic inflammatory disorders. Previously in [6] was demonstrated significant implication of the metastasis-inducing S100A4 in the pathogenesis of rheumatoid arthritis, psoriasis and dermato/polymyosites [7]. We suggested that the significance of the S100 proteins, as a diagnostic and therapeutic targets, could be pivotal in particular inflammatory diseases and proposed to study their role in FMF.

First we chose to analyze the levels of S100A4 in plasma of FMF patients at different activity phases of FMF. The reason of our first choice was that:

(i) we have shown strong activation of S100A4 in other autoimmune inflammations;

(ii) we have discovered that S100A4 strongly activate expression of various cytokines (TNF-α, RANTES, G-CSF and others) and Serum Amyloid A (a circulating precursor of AA amyloid) in various mouse organs upon systemic, intravenous application (manuscript submitted);

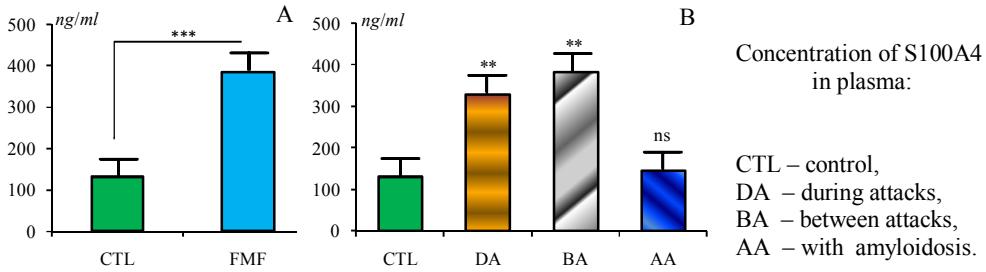
(iii) S100A4 is highly expressed in neutrophils, whose massive influx into serous cavities is a characteristic feature for FMF attacks.

Materials and Methods. Plasma samples from 100 children and adolescents with a clinical and/or genetic diagnosis of FMF and 25 healthy persons were collected in CMG, Yerevan and transported to DCS, Copenhagen for analysis. Plasma samples were categorized into three groups according to the clinical activity of FMF: between attacks, during attacks and patients with amyloidosis.

Samples were analyzed using (i) S100A4-specific sandwich ELISA developed in DCS using S100A4-specific poly- and monoclonal antibodies raised in DCS; (ii) Western blot analysis of plasma samples for correlation of S100A4 and SAA levels and their conformational forms.

Results and Discussion.

• First we have established the optimal conditions for S100A4-specific sandwich ELISA. To avoid rather possible aggregation and sedimentation of S100 and SAA proteins we add to the freshly collected plasma samples from five patients and healthy controls one the following agents: Triton-X100, NP-40, Arginine or PBS. The data demonstrated that the used agents do not influence the efficiency of S100A4 detection in ELISA. Therefore the following experiments were continued without prior manipulation of the plasma samples.



• Level of S100A4 has been determined in three pilot experiments: plasma collections including patients at various clinical activity stages of FMF and control persons without clinical signs of FMF.

Data obtained in 36 FMF patient indicated a highly significant increase of S100A4 proteins level in patients' plasma compared to healthy non-FMF persons (Figure A).

• Next we analyzed groups of patients at various phases of clinical activity of FMF such as during attacks (DA), between attacks (BA) and FMF patients with developed amyloidosis (AA) (n=7–10). The data obtained in the pilot experiment revealed significantly high plasma levels of S100A4 in DA and BA and lower S100A4 levels in patients with the diagnosed amyloidosis (Figure B).

We have examined the correlation between the S100A4 and SAA content in control (n=3) and FMF (n=7) using Western blot analysis of plasma samples. Our preliminary data indicate the association of an increased level of SAA with a modified conformational form of S100A4 identified as an additional higher molecular protein band.

• We found a tendency correlating genotype of FMF patients with the increased levels of S100A4 in plasma. Thus, in out of 36 FMF patients 22 (61%) revealed more than 300 *ng*/*ml* of S100A4 in plasma and 15 (68%) of them were carriers of mostly compound heterozygote mutations, mainly M694V and V726A, whereas among 14 (39%) patients with less than 300 *ng*/*ml* S100A4 only 5 (35%) were carriers of heterozygote mutations in pyrin.

• Studies on another member of the S100 family members (S100A7) in FMF patients (n=10) revealed no reactivity for this protein in plasma samples.

Conclusions.

1. S100A4 might be a valuable biomarker for monitoring FMF activity. It might even sense subclinical inflammation in patients without febrile attacks.

2. Specific conformational forms of S100A4 (need to be identified) also might have significance in the pathogenesis of FMF and particularly in its deadly complication, AA amyloidosis.

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