A72 **ROLE OF THE PRO-APOPTOTIC PYRIN-INTERACTING PROTEIN SIVA IN THE PATHOGENESIS OF FAMILIAL MEDITERRANEAN FEVER**

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Background Familial Mediterranean fever (FMF) is an autosomal recessive disease attributed to mutations in the MEFV gene encoding pyrin, which is characterised by recurrent, acute and self-limiting attacks of fever. Most disease-associated mutations in the MEFV gene reside on the C-terminal PRYSPRY (B30.2) domain of pyrin, an area found to interact with the pro-apoptotic protein Siva.

Aim (1) To define the topology of the (full-length) Siva-1 with respect to its interaction with pyrin and other interacting proteins; (2) to investigate the ability of mutant forms of pyrin to interact with Siva-1; and (3) to explore the potential increase of GTR/Siva-induced apoptosis in the presence of the mutant forms of pyrin.

Materials and Methods A three-dimensional model of Siva-1 was created with MODELLER and a Swiss model automated protein structure homology-modelling server. HEK293 (human embryonic kidney) and THP-1 (human monocytic leukaemia) cell lines were used for transfection experiments using Lipofectamine 2000 or the Amaxa nucleofection technology, respectively. Apoptosis was measured by FACS-Annexin-PE staining using the Annexin-V kit (Pharmingen).

Results The authors constructed a three-dimensional model of Siva-1 and three distinct domains were defined, an N-terminal domain containing amphipathic helices with potential to interact with the PRYSPRY domain of pyrin, a B-box-like domain and a zinc finger-like C-terminal domain that the authors assume to bind the cytoplasmic tail domain of GTR protein. In immunoprecipitation experiments on transfected HEK293 cells, Siva bound both to wild-type pyrin and its mutant forms, but no differences were observed in the rates of apoptosis in THP-1 upon transfection with either wild-type pyrin or mutant forms of pyrin. Patients with FMF did not display any mutations in the Siva gene.

Conclusions The three-dimensional model of Siva-1 constructed showed that GTR and pyrin do not antagonise for the same binding site(s). The data obtained by using various mutants of pyrin and Siva-1 for cotransfecting HEK293 cells suggest that the positions of the PRYSPRY domain, found mutated in FMF patients, are not required for binding to Siva-1. The authors were not able to demonstrate an increase in GTR/Siva-induced apoptosis in the presence of the mutant

forms of pyrin, thus suggesting that Siva is unlikely to contribute to the pathogenesis of FMF through the postulated molecular mechanism.