

## Tumour Necrosis Factor Receptor-associated Periodic Syndrome (TRAPS): Identification of Novel TNFRSF1A Mutations and Intracellular signalling Defects

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Susanna Stjernberg-Salmela, MD, defended her PhD thesis on 29 August 2008 in Helsinki. The opponent was Professor Joost Frenkel from the Department of Pediatrics, University of Utrecht, The Netherlands. The thesis was supervised by Professor Annamari Ranki from the Department of Dermatology, and Associate Professor Tom Pettersson from the Department of Medicine, University of Helsinki, Finland. The thesis book is available at: <http://urn.fi/URN:ISBN:978-952-10-4848-7>.

The systemic auto-inflammatory disorders are a group of rare diseases characterized by periodically recurring episodes of acute inflammation and a rise in serum acute phase proteins, but with no signs of autoimmunity. At present 8 hereditary syndromes are categorized as auto-inflammatory, although the definition has also occasionally been extended to other inflammatory disorders, such as Crohn's disease. One of the auto-inflammatory disorders is the autosomally dominantly inherited tumour necrosis factor receptor-associated periodic syndrome (TRAPS), which is caused by mutations in the gene encoding the tumour necrosis factor (TNF) type 1 receptor (TNFRSF1A). In patients of Nordic descent, cases of TRAPS and of three other hereditary fevers (hyperimmunoglobulinaemia D with periodic fever syndrome (HIDS); chronic infantile neurological, cutaneous and articular syndrome (CINCA); and familial cold auto-inflammatory syndrome (FCAS)) have been reported, TRAPS being the most common of the four. The clinical characteristics of TRAPS are recurrent attacks of high spiking fever, associated with inflammation of serosal membranes and joints, myalgia, migratory rash and conjunctivitis or periorbital cellulitis. Systemic amyloid A (AA) amyloidosis may occur as a sequel of the systemic inflammation.

The aim of this study was to investigate the genetic background of hereditary periodically occurring fever syndromes in Finnish patients, to explore the reliability of determining serum concentrations of soluble TNFRSF1A and metalloproteinase-induced TNFRSF1A shedding as helpful tools in differential diagnostics, and to study intracellular NF- $\kappa$ B signalling in an attempt to widen the knowledge of the pathomechanisms underlying TRAPS.

Genomic sequencing revealed two novel *TNFRSF1A* mutations, F112I and C73R, in two Finnish families. F112I was the first *TNFRSF1A* mutation to be reported in the third extracellular cysteine-rich domain of the gene and C73R was the third novel mutation to be reported in a Finnish family, with only one other *TNFRSF1A* mutation having been reported in the



Fig. 1. Susanna Stjernberg-Salmela with the opponent Professor Joost Frenkel from Utrecht and the thesis supervisor Professor Annamari Ranki after the thesis defence on 29 August 2008.

Nordic countries. We also presented a differential diagnostic problem in a TRAPS patient, emphasizing for the clinician the importance of differential diagnostic vigilance in dealing with rare hereditary disorders. The underlying genetic disease of the patient both served as a misleading factor, which possibly delayed the correct diagnosis, but may also have predisposed to the pathological condition, which led to a critical state of the patient. Using a flow cytometric analysis method modified for use on fresh whole blood, we studied intracellular signalling pathways in three Finnish TRAPS families with the F112I, C73R and the previously reported C88Y mutations. Evaluation of (TNF)-induced phosphorylation of NF- $\kappa$ B and p38, revealed low phosphorylation profiles in nine out of ten TRAPS patients in comparison with healthy control subjects.

This study shows that TRAPS is a diagnostic possibility in patients of Nordic descent, with symptoms of periodically recurring fever and inflammation of the serosa and joints. In

particular, in the case of a family history of febrile episodes, the possibility of TRAPS should be considered if an aetiology of autoimmune or infectious nature is excluded. The discovery of three different mutations in a population as small as the Finnish, reinforces the notion that the extracellular domain of *TNFRSF1A* is prone to be mutated at the entire stretch of its cysteine-rich domains and not only at a limited number of sites, suggesting the absence of a founder effect in TRAPS. This study also demonstrates the challenges of clinical work in differentiating the symptoms of rare genetic disorders from those of other pathological conditions, and presents the possibility of an auto-inflammatory disorder as being the underlying cause of severe clinical complications. Furthermore, functional studies of fresh blood leukocytes show that TRAPS is often associated with a low NF- $\kappa$ B and p38 phosphorylation profile, although low phosphorylation levels are not a requirement for the development of TRAPS. The aberrant signalling profile would suggest that the hyperinflammatory phenotype of TRAPS is the result of compensatory NF- $\kappa$ B-mediated regulatory mechanisms triggered by a deficiency of the innate immune response.

### List of original publications

The thesis is based on the following original communications, referred to in the text as Roman numerals:

- I. Nevala H, Karenko L, Stjernberg S, Raatikainen M, Suomalainen H, Lagerstedt A, et al. A novel mutation in the third extracellular domain of the tumor necrosis factor receptor 1 in a Finnish family with autosomal-dominant recurrent fever. *Arthritis Rheum* 2002; 46: 1061-1066.
- II. Stjernberg-Salmela S, Pettersson T, Karenko L, Blazevic V, Nevala H, Pitkänen S, Peterson P, Ranki A. A novel tumour necrosis factor receptor mutation in a Finnish family with periodic fever syndrome. *Scand J Rheumatol* 2004; 33: 140-144.
- III. Stjernberg-Salmela S, Kivisaari A, Puolakkainen P, Färkkilä M, Kempainen E, Ranki A, Pettersson T. Intra-abdominal abscess in a patient with tumour necrosis factor receptor-associated periodic fever syndrome. *J Intern Med* 2006; 259: 209-213.
- IV. Stjernberg-Salmela S, Ranki A, Karenko L, Siitonen S, Mustonen H, Puolakkainen P, Sarna S, Pettersson T, Repo H. Low TNF-induced NF- $\kappa$ B and p38 phosphorylation levels in leukocytes in tumour necrosis factor receptor-associated periodic syndrome (TRAPS). Submitted.

In addition this thesis contains some previously unpublished data.

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## Topical Tacrolimus in Atopic Dermatitis: Effects of Long-term Treatment on Skin and Respiratory Symptoms

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Dr Hannele Virtanen from the Department of Dermatology, Allergology, and Venereology, University of Helsinki, Finland, defended her PhD thesis on 25 February 2008 in Helsinki. The opponent was Professor Thomas Ruzicka from the Ludwig-Maximilians University, Munich, Germany. The thesis was supervised by Docent Sakari Reitamo. The thesis publication is available at: <http://urn.fi/URN:ISBN:978-952-10-4520-2>.

Atopic dermatitis (AD) is a chronic pruritic skin disease often complicated by *Staphylococcus aureus* colonization and superinfection. In patients with AD, concomitant occurrence of other atopic diseases, such as asthma and allergic rhinoconjunctivitis, is common. Skin atrophy is the major problem in conventional corticosteroid-based treatment of AD, which limits treatment to short periods, especially in areas of thin skin. After the treatment period, AD often relapses. In practice, mainly the patients with mild disease achieve a longer remission with periodic short-term use of topical corticosteroids. Patients with moderate or severe AD are usually poorly controlled with short-term treatments and experience continuous skin inflammation of varying degrees. The second-line treatments, such as ultraviolet light therapy, cyclosporine, and azathioprine, diminish the need for topical

corticosteroids but do not replace them. The barrier function of the inflamed skin is weakened, possibly enabling an epicutaneous sensitization to occur, which may lead to atopic respiratory disease via systemic T-cell responses and airway hyper-responsiveness. The need has therefore been great for a non-atrophogenic treatment option, especially for patients with moderate-to-severe AD requiring long-term treatment.

Although AD is a very common and chronic disease, efficacy in placebo-controlled studies has been shown only for topical corticosteroids, oral cyclosporine, and azathioprine. Furthermore, only a few long-term studies involve topical corticosteroids. Tacrolimus ointment belongs to the group of topical immunomodulators, which have a more specific mode