Doctoral Thesis

Heat shock protein 65 of Tropheryma whippelii molecular characterization, phylogenetic analysis and detection of antibodies in patients

Author(s):
Morgenegg, Silvia

Publication Date:
2001

Permanent Link:
https://doi.org/10.3929/ethz-a-004225138

Rights / License:
In Copyright - Non-Commercial Use Permitted
Heat shock protein 65 of *Tropheryma whippelii*: molecular characterization, phylogenetic analysis and detection of antibodies in patients

A dissertation submitted to the
Swiss Federal Institute of Technology Zürich
for the degree of
Doctor of Natural Sciences

presented by

Silvia Morgenegg

Pharmacist (Eidg. dipl. Apothekerin)
ETH Zürich

born May 8th, 1969
citizen of Köniz, Switzerland

accepted on the recommendation of

Prof. Dr. G. Folkers, examiner
Prof. Dr. M. Altwegg, co-examiner
Prof. Dr. A. von Graevenitz, co-examiner

2001
SUMMARY

Whipple’s disease is a systemic infection of humans associated with a wide variety of intestinal as well as extraintestinal manifestations. The causative agent, *Tropheryma whippeli*, has so far never been cultivated on artificial media. The molecular detection of this rare bacterium is currently based on PCR-mediated amplification of a part of its 16S ribosomal RNA gene (16S rDNA), of the 16S-23S internal transcribed spacer (ITS), or of the 23S rDNA.

Recently, *T. whippeli* DNA has been found in duodenal biopsies and gastric juice of patients without Whipple’s disease. In a follow-up study, saliva and dental plaques of the same patients were screened for the presence of *T. whippeli* DNA (see Chapter 2). Six out of the 14 previously PCR-positive persons but none of the 17 controls had *T. whippeli* DNA in their saliva (the same ITS-type). Our results suggest that Whipple bacteria are ubiquitous environmental or commensal organisms causing Whipple’s disease in a particular subset of individuals, possibly those with an as yet uncharacterized immunological defect.

We wanted to confirm these results with a target completely different from the rRNA genes used in all these studies. The heat shock protein 65 (Hsp65) and the gene coding for it (*hsp65*) are ubiquitous and therefore most probably also found in *T. whippeli*. Using broad-spectrum primers, we have amplified, cloned and sequenced a 620 bp-fragment of the *T. whippeli* heat shock protein (*hsp65*) gene from the heart valve of a patient with Whipple’s endocarditis (see Chapter 3). With primers derived from this sequence a semi-nested PCR was established. Seventeen patients with or without clinical signs of Whipple’s disease shown to be positive by 16S rDNA-PCR and/or ITS-PCR were also positive by *hsp65*-PCR. Clinical samples from 33 control patients remained negative. We conclude that the *hsp65*-PCR represents a sensitive and specific alternative assay for the molecular diagnosis of Whipple’s disease.

Only recently, Raoult and coworkers were able to cultivate *T. whippeli* in a human cell line. With bacterial DNA isolated from these cell cultures and with primers derived from the previously determined 620 bp-fragment, we determined the unknown up- and downstream regions of the *hsp65* gene of *T. whippeli* by genome walking (see Chapter 4). After determination of the entire gene sequence (1623 bp), the corresponding amino acid sequence provided a basis for evolutionary comparisons. The phylogenetic analysis obtained showed higher bootstrap values than those from the 16S rDNA data set and confirmed that *T. whippeli* is indeed a member of the Gram-positive bacteria with high G + C content (actinobacteria). Only slight differences to trees based on 16S rDNA gene sequences were observed.
Recombinant plasmids carrying the partial and the entire *T. whippelii hsp65* gene were constructed, and expression yielded recombinant proteins of 32.8 and 65 kDa, respectively (see Chapters 5 and 6). Immunoblot analysis revealed only weak antibody response. The entire Hsp65 was then purified by affinity chromatography and used in a quantitative
ZUSAMMENFASSUNG

(Aktinobakterien) gehört. Es wurden nur kleine Differenzen zu den 16S rDNA-Stammbäumen beobachtet.
Rekombinante Plasmide, die das partielle und ganze T. whipplei hsp65-Gen tragen, wurden konstruiert. Die Expression ergab Proteine von 32,8 respektive 65 kDa (Kapitel 5 and 6). Im Immunoblot zeigte sich nur eine schwache Antikörper-Antwort. Das ganze Hsp65 wurde mittels Affinitätschromatographie gereinigt und in einem Enzyme-linked