Gene expression based molecular test proves clinical validity as diagnostic aid for the differential diagnosis of psoriasis and eczema in formalin fixed and paraffin embedded tissue

Felix Fischer[1*], Anais Doll[2*], Deniz Uereyener[1], Sophie Roenneberg[2], Christina Hillig[1], Lucca Weber[3], Verena Hackert[3], Peter Seiringer[2], Jenny Thomas[2], Philipp Anand[2], Larissa Graner[5], Franziska Schlenker[5], Roland Zengerle[4,5], Manja Jargosch[2,6], Fabian J. Theis[1,7], Carsten B. Schmidt-Weber[6], Tilo Biedermann[2], Michael Howell[8], Kristian Reich[9], Michael Menden[1], Natalie Garzorz-Stark[2], Felix Lauffer[2*], and Stefanie Eyerich [6,*]

[1]Institute of Computational Biology, Helmholtz Center Munich, Neuherberg, Germany; [2]Department of Dermatology and Allergy, Technical University of Munich, Munich, Germany; [3] Dermagnostix GmbH, Friedberg, Germany; [4]Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, [5]University of Freiburg, Freiburg, Germany, Hahn-Schickard Society for Applied Research, Freiburg, Germany;[6]ZAUM – Center of Allergy and Environment, Technical University of Munich and Helmholtz Center Munich, Member of the German Center for Lung Research (DZL), Munich, Germany; [7]Department of Mathematics, Technical University of Munich, Garching, Germany; [8]DermTech, La Jolla, California, United States of America

Introduction

Highly specific and efficient drugs have been developed to treat non-communicable chronic inflammatory skin diseases (ncISD). Due to their specificity, these drugs require precise diagnostics to attribute the most efficient treatment to each patient. Diagnosis is complicated by the complex pathogenesis of ncISD and their clinical and histological overlap. Especially, precise diagnosis of psoriasis and eczema is difficult in special cases and molecular tools need to be developed to support gold standard diagnosis. A gene expression-based classifier using NOS2 and CCL27 has been proposed and its clinical validity examined and proven in various patient cohorts.

Objectives

To develop a real-time based molecular classifier (MC) to distinguish psoriasis from atopic eczema in FFPE-fixed skin samples for diagnostic measures and to evaluate the potential of minimally invasive microbiopsies and non-invasive tape strips for molecular diagnosis.

Methods

FFPE, micro-(Ø1mm) and macrobiopsies (Ø4-6mm), and tape strips were collected from psoriasis and eczema lesions and analyzed by real-time PCR for the expression of NOS2 and CCL27. A molecular classifier (MC) was established using a linear regression model.

Conclusions

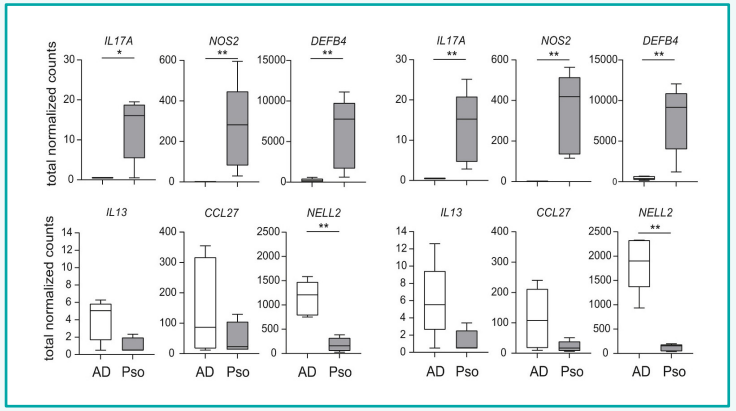
The FFPE-based molecular classifier (MC) precisely separates eczema from psoriasis and efficiently identifies subtypes of both diseases, facilitating a possible implementation of this molecular diagnostic aid in routine clinical pathological practice.

We further show that the gene expression profile of RNA later fixed microbiopsies is comparable to standard 4-6mm biopsies and that microbiopsies are equally suited for the MC. We demonstrate the potential of tape strips of inflamed epidermis for molecular diagnostics of ncISD. Due to the minimally invasive sampling procedure, tape stripping may be particularly useful for the testing of visible and sensitive skin areas and in children as well as for repetitive sampling procedures necessary to monitor treatment responses over time.

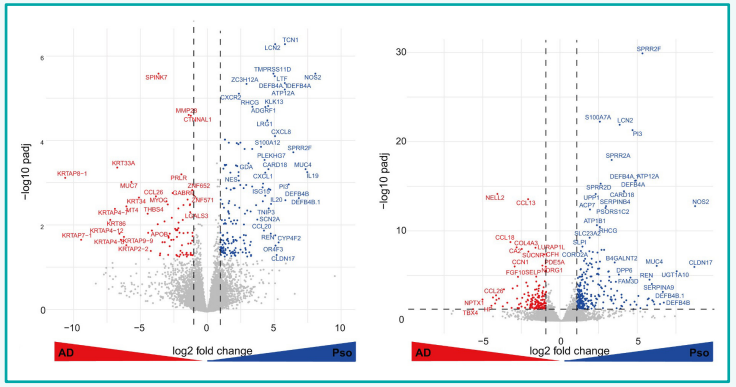
In summary, the MC discriminates psoriasis from eczema producing meaningful results in a broad range of skin tissue derived from invasive to minimally invasive sampling techniques. Further efforts are required to simplify and shorten the labor- and cost-intensive procedures of RNA isolation and real-time PCR. One possible solution is a fully automated and closed system which would allow the integration of molecular diagnostics into the routine patient management at the point of care.

Results

The FFPE-based classifier determined probabilities for psoriasis with a sensitivity and specificity and of 92% and 100%, respectively, and an AUC of 0.97. To test if microbiopsies are also adequate tissue samples for the MC, we analyzed gene expression in 83 pairs of macro- and microbiopsies by qRT-PCR. Delta-CT values showed no significant difference between macro- and microbiopsies. Delta Ct values of NOS2 and CCL27 also efficiently separated psoriasis from eczema samples in inflamed skin collected via tape strips.



Micro (R)- and macrobiopsies (L) show comparable gene expression profiles enabling the use of microbiopsies for molecular diagnosis. Hallmark genes for AD (white) and Pso (gray) pathogenesis.



Volcano plot of significantly regulated genes (Padj < 0.05, log2foldchange > 1) in macrobiopsies and microbiopsies determined by RNA sequencing