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ORIGINAL PAPER

Ex vivo nonlinear microscopy imaging of Ehlers–Danlos syndrome-affected skin

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Abstract
Ehlers–Danlos syndrome (EDS) is the name for a heterogenous group of rare genetic connective tissue disorders with an overall incidence of 1 in 5000. The histological characteristics of EDS have been previously described in detail in the late 1970s and early 1980s. Since that time, the classification of EDS has undergone significant changes, yet the description of the histological features of collagen morphology in different EDS subtypes has endured the test of time. Nonlinear microscopy techniques can be utilized for non-invasive in vivo label-free imaging of the skin. Among these techniques, two-photon absorption fluorescence (TPF) microscopy can visualize endogenous fluorophores, such as elastin, while the morphology of collagen fibers can be assessed by second-harmonic generation (SHG) microscopy. In our present work, we performed TPF and SHG microscopy imaging on ex vivo skin samples of one patient with classical EDS and two patients with vascular EDS and two healthy controls. We detected irregular, loosely dispersed collagen fibers in a non-parallel arrangement in the dermis of the EDS patients, while as expected, there was no noticeable impairment in the elastin content. Based on further studies on a larger number of patients, in vivo nonlinear microscopic imaging could be utilized for the assessment of the skin status of EDS patients in the future.

Keywords Ehlers–Danlos syndrome · Nonlinear microscopy · Second-harmonic generation · Collagen structure

Introduction

Ehlers–Danlos syndrome (EDS) is the name for a heterogeneous group of rare genetic connective tissue disorders with an overall incidence of 1 in 5000 [2]. EDS was first recognized as a distinct entity in 1901 by Edvard Lauritz Ehlers, a Danish physician who published a description of a patient with hyperextensible skin, hypermobility of joints and a tendency for bruising. A further case was reported in 1908 by Henri-Alexandre Danlos, a French dermatologist. In 1949, Falls and Johnson deduced that EDS follows an autosomal dominant (AD) pattern of inheritance. The first molecular alteration, identifying lysyl hydroxylase deficiency in the background of an autosomal recessive (AR) form of EDS was reported in 1972 [23]. Several EDS classifications have been proposed since the 1960s. The "Berlin Nosology", published in 1988 defined 11 EDS subtypes based on patterns of inheritance and clinical presentation [1]. Following the description of the genetic and molecular basis of several subtypes, a revised, simplified classification, the "Villefranche Nosology" was released in 1997 and has since been widely used for two decades. In this classification, 6 EDS subtypes were distinguished and for each type minor and major criteria were defined. Since the publication of the "Villefranche Nosology", with the development of next-generation sequencing (NGS) techniques various novel mutations were identified and new EDS subtypes have been introduced [2]. The current, updated international classification of EDS was published recently, in 2017 [18]. The new nosology delineates 13 clinical subtypes and defines their

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