# Dermal ultrastructure in a case of Parry-Romberg syndrome

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## SUMMARY

A case of Parry Romberg syndrome (PRS) in a 7-year-old girl is described. Ultrastructural investigations, supported by clinical evaluations, were performed on both affected and unaffected skin. Connective tissue abnormalities were mainly observed in the diseased area, where an increased number of mast cells can be observed, and collagen is organized in large bundles with fibrils of heterogeneous diameters. Data are suggestive for increased matrix remodeling in the affected skin.

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## Introduction

Parry-Romberg syndrome (PRS), also known as progressive facial hemiatrophy, is a rare disease characterized by facial tissue atrophy, more frequently occurring unilaterally and involving skin and subcutaneous tissue (Whyman *et al.,* 1992; Stone, 2006; Duymaz *et al.,* 2009; Patel *et al*., 2010). The disease was firstly described by Caleb Parry in 1825 and by Moritz Romberg in 1846, but, still, the etiology and the pathogenic mechanisms leading to the clinical phenotype are unknown. Autoimmunity, viral infection, dysfunction of the nervous system, endocrine disorders have been hypothesized as potential causative events (Baskan *et al.,* 2006; Budrewicz *et al.,* 2012; Bucher *et al.,* 2016). The prevalence of the disease has been estimated around 1:700.000, women are slightly more affected than man with a ratio of 3:2 (Lakhani and David, 1984; Miller *et al.,* 1987; Stone, 2006) and disease onset usually starts during the first or second decade of life, slowly progressing for a number of years possibly affecting also the underlying muscles, cartilage and/or bone (Stone, 2003; Paprocka *et al.,* 2006). The clinical phenotype is characterized by a great heterogeneity: some patients exhibit a prevalence of neurologic disorders, whereas others are mainly characterized by connective tissue alterations,

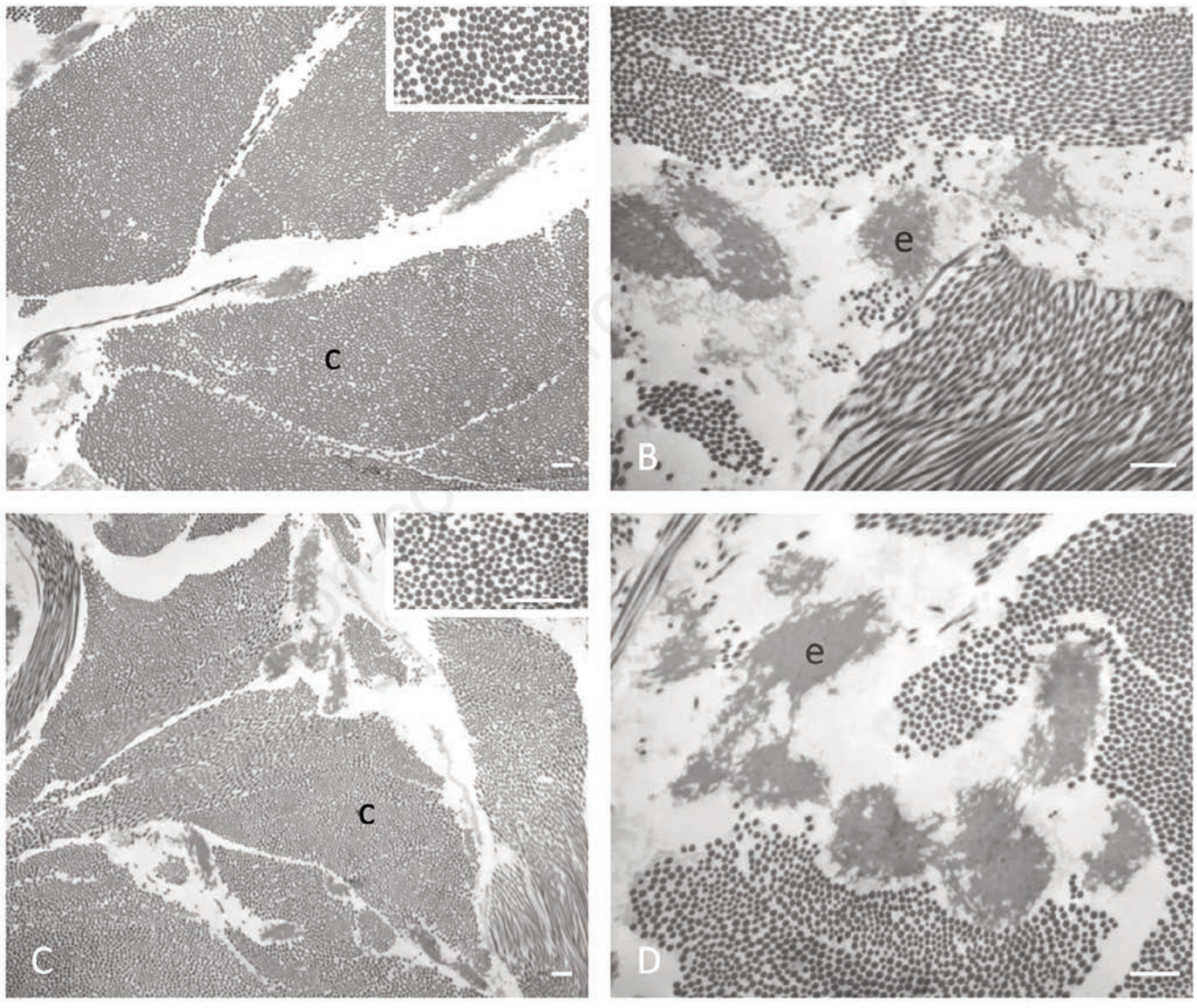


**Figure 1. Dermal ultrastructure of unaffected (A-B) and affected (C-D) skin. Collagen (c) bundle organization is shown in panels A and C, whereas the frayed contour of elastic fibers (e) is better visualized at higher magnification in panels B and D. The diameter of collagen fibrils is clearly visible in inserts. Scale bars: 1 µm.**

thus suggesting that PRS can represent a clinical entity largely overlapping a localized form of scleroderma, *i.e*. linear scleroderma or en coupe de sabre, as part of the same spectrum of diseases (Peterson *et al*., 1995; Laxer and Zulian, 2006; Khamaganova, 2018).

Despite connective tissue involvement, very few data are available on dermal structural organization in PRS. Previous investigations have demonstrated that diseased specimens have increased levels of glycosaminoglycans and/or hyaluronic acid, without any change in the collagen content compared to controls (Sakuraoka *et al.*, 1992), but to our knowledge comparison between affected and unaffected areas was never done. The present study, for the first time, describes a case of PRS affecting a young girl, focusing on the ultrastructure of the dermis in both affected and unaffected skin.

## Case Report

A 7-year-old girl presented progressive facial hemiatrophy on the left side of her face since the age of 5. Moreover, a dimple region in the left parietal bone and axonometric palate associated with dental malocclusion were also observed, consistent with skeletal findings frequently observed in the mandibula (Wong *et al*., 2015). Skin appeared thin with reduced elasticity and local alopecia. Neurologic investigations did not highlight any abnormalities of cerebral structure and function.

Skin biopsies were obtained from left and right side of the scalp corresponding to affected and unaffected areas, respectively. Samples were immediately fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer and processed for electron microscopy as already described (Boraldi *et al.,* 2019). Ultrathin sections were observed

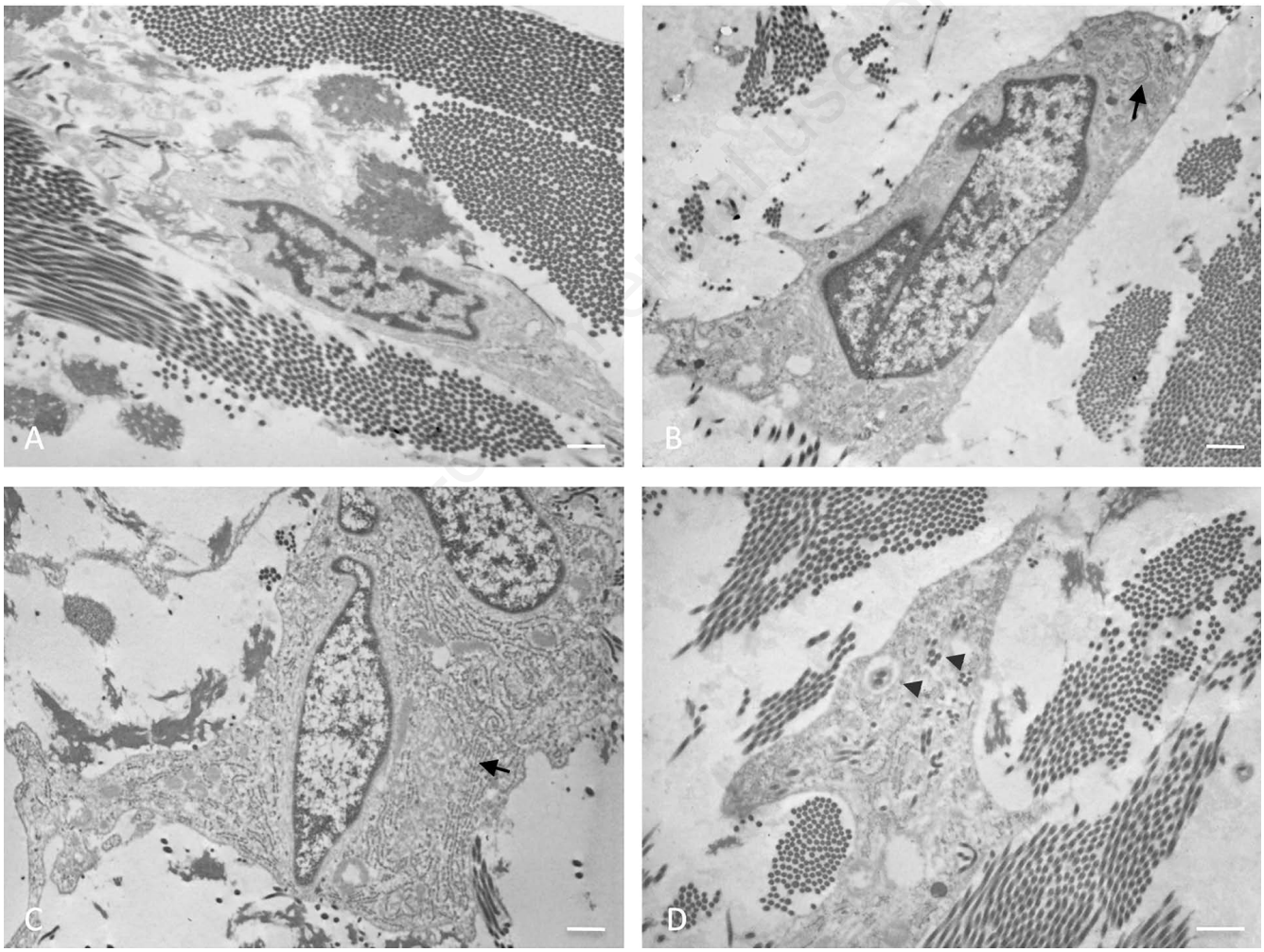


**Figure 2. Dermal fibroblasts in the dermis of unaffected (A-B) and affected (C-D) skin. The abundance of the endoplasmic reticulum (arrows) and the presence of collagen fibrils within cytoplasmic vesicles (arrowheads) are frequently observed in cells in the diseased dermis. Scale bars: 1 µm.**

with a SEM-FEG FEI Nova450 (ThermoFisher Scientific, Waltham, MA, USA). Procedures were in accordance with the basic principles of the Declaration of Helsinki and written informed consent was obtained by the patient's parents.

## Ultrastructural findings

Ultrastructural observations of clinical unaffected dermis revealed numerous collagen bundles comprised of fibrils with homogeneous diameters (Figure 1A and insert). Elastic fibers were rather small, polymorphic and with an irregular contour (Figure 1B) exhibiting a number of cisternae of the holes within the amorphous core. At the fibers' periphery accumulation of unstructured electron-dense material can be observed, suggestive for the presence of microfibrillar associated glycoproteins uncovered by tropoelastin molecules.

In clinically affected areas, the dermis revealed large collagen bundles comprised of less compact fibrils with heterogeneous diameters (Figure 1C and insert). Elastic fibers, compared to those in patient's healthy skin, were similarly small, but highly frayed (Figure 1D). The microfibrillar network was barely detectable.

The ultrastructural phenotype of dermal fibroblasts is shown in Figure 2. In clinical unaffected areas, fibroblasts exhibit an elongated shape in close contact with elastin fibers and collagen fibrils. Cisternae of the endoplasmic reticulum are visible (Figure 2 A,B). Fibroblasts in the affected skin were characterized by cytoplasm filled with abundant and densely packed endoplasmic reticulum (Figure 2C). Interestingly, a number of fibroblasts exhibited collagen fibrils within vacuoles and membrane surrounded vesicles (Figure 2D).

Moreover, in clinically affected skin, interactions between fibroblasts and mast cells can be frequently observed (Figure 3). In these areas the extracellular matrix was less organized, collagen bundles being smaller with scattered fibrils.

## Discussion

Perry Romberg Syndrome is characterized by neurologic and/or connective tissue alterations ranging from atrophy (Wong *et*

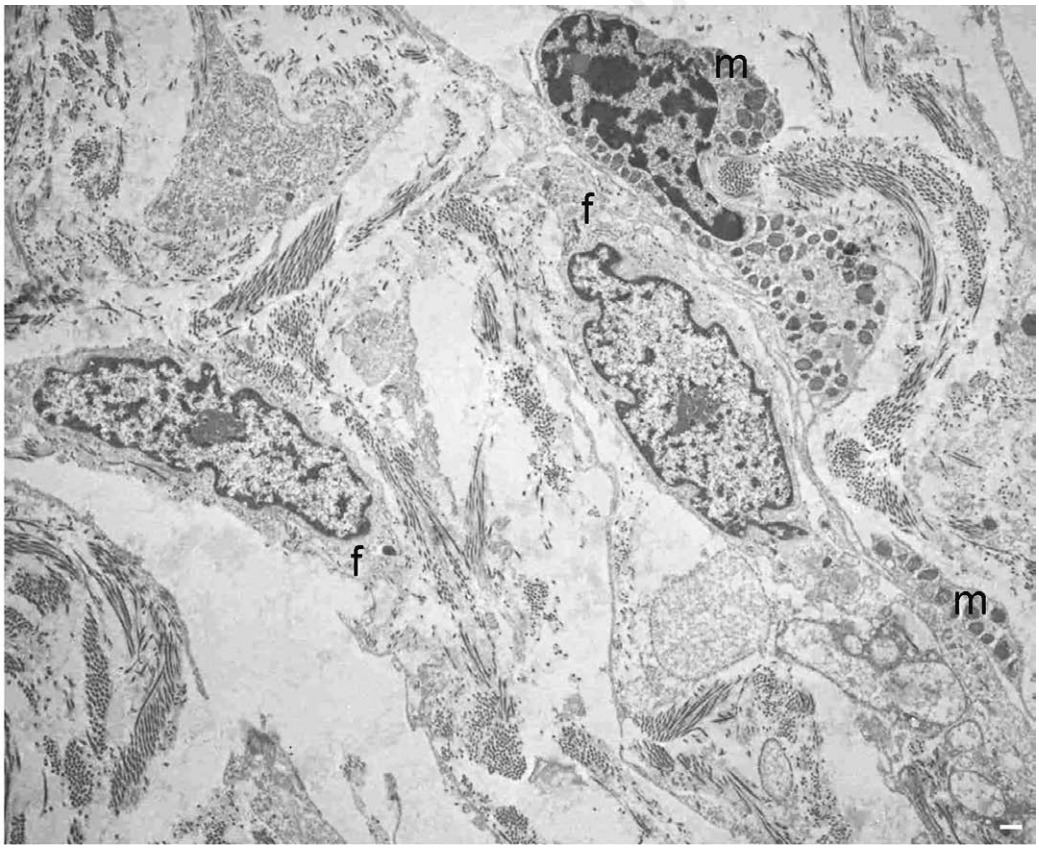


**Figure 3. Dermal ultrastructure of clinically affected skin highlighting the frequent interactions between fibroblasts (f) and mast cells (m). Scale bars: 1 µm.**

*al.,* 2015) to fibrosis often overlapping that typical of a scleroderma/scleroderma-like clinical phenotype (Paprocka *et al.,* 2006; Tollefson and Witman, 2007). The heterogeneity of the clinical phenotype makes PRS diagnosis rather challenging and therefore it has been suggested that PRS, linear scleroderma and en coupe de sabre may represent a spectrum of the same diseases (Khamaganova, 2018; Schultz *et al.,* 2019).

Therefore, in the absence of a clear genetic signature (Chen *et al.,* 2018), diagnosis is still based on clinical findings (Schultz *et al,.* 2019).

In the light of few data reported in the literature, connective tissue alterations in PRS seem to be limited to altered synthesis of glycosaminoglycans such as dermatan sulfate and hyaluronic acid, without any involvement of collagen and elastin (Wong *et al.,* 2015).

By ultrastructural observations in both affected and unaffected dermis of the same patient, results indicate that clinical features are associated with a significant matrix remodeling as demonstrated by the heterogeneity of collagen fibril diameters, the abundance of fibroblasts endoplasmic reticulum and the presence of collagen fibrils within cytoplasmic vacuoles. This last feature has been suggested to represent a sign of active procollagen processing (Canty and Kadler, 2005), being consistent with increased matrix remodeling. These findings are also in agreement with the more frequent occurrence of mast cells in the affected dermis. Interestingly, mast cells have been involved in the pathogenesis of fibrotic processes, including systemic sclerosis and scleroderma (Atkins *et al.,* 1985; Ozbilgin and Inan, 2003; Arbi *et al.,* 2015; Bradding and Pejler, 2018). Mast cells can store and release a number of lipid-derived mediators, growth factors and cytokines as well as serine proteases such as tryptase and chymase. Tryptase induces fibroblasts migration and proliferation as well as collagen synthesis, whereas chymase can promote fibrosis via the transforming growth factor (TGF)-β1/Smads signaling pathway (Chen *et al.,* 2017). Moreover, since mast cells can influence angiogenesis as well as elastin degradation (Sun *et al.,* 2009; Wang and Shi, 2012), these data further highlight the importance of the crosstalk between fibroblasts and mast cells (Zhang *et al.,* 2011), suggesting that these interactions may be involved also in dermal connective tissue alterations in Parry Romberg syndrome.

Finally, the observation that abnormalities are restricted to specific sites and that dermal connective tissue characteristics exhibit differences at the ultrastructural level between affected and unaffected dermis, further supports the hypothesis that exogenous factors may trigger a local response with detrimental effects on connective tissue homeostasis. These findings, together with the observation that disease progression can stop after a number of years, without any apparent reason (Wong *et al.,* 2015), can open new perspectives for the appropriate timing of tissue reconstruction procedures (Chen *et al.,* 2018).

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