miR-29a at the SSD stage, but maintained by other factors in SSc patients.

Table 1a shows the association of serum miR-29a levels with the clinical features in SSc patients. Considering that collagen expression in SSc patients is up-regulated as described above, we regarded reduction of miR-29a levels as the meaningful change. We found that SSc patients with reduced miR-29a levels had significantly higher right ventricular systolic pressure by Doppler echocardiography than those with normal levels. Though the cause of pulmonary hypertension in SSc is still uncertain, our results suggested that miR-29a also takes part in the pathogenesis of pulmonary hypertension. On the other hand, as shown in Table 1b, clinical features of each SSD patient were not correlated with the levels of miR-29a. Taken together, serum miR-29a levels is not likely to be a specific marker for clinical manifestations of SSD, but the miRNA may play an important role in the pathogenesis of this disease.

The concept of SSD has been proposed by Maricq et al. originally to unify typical SSc, early forms of SSc and closely related disorders including mixed connective tissue disease (MCTD) [8,9]. Thereafter, Ihn et al. established a new diagnostic method using a points system to distinguish patients with SSD from those with early SSc [10]. Although the point system has not been accepted in the world wide basis, because progressive fibrosis of SSc is often irreversible, at least clinically, there is an urgent need to develop new strategies to diagnose patients as early as possible and follow-up carefully. For that purpose, the concept of SSD should be further understood and characterized. Our study is the first to examine serum miRNA levels using sera from SSD as well as SSc, and is shedding new light on the definition of SSD. To note, it may be difficult to distinguish early stage SSc from SSD, because skin sclerosis is sometimes not apparent in early SSc, especially in lcSSc. Serum levels of miR-29a levels may be useful for the differentiation of SSc from SSD.

As the limitation of this study, we could not collect large number of SSD patients because of the rarity of this condition. However, our approach may be effective to clarify the property of SSD. Larger studies are needed in the future.

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Letter to the Editor

Differential hyaluronan homeostasis and expression of proteoglycans in juvenile and adult human skin

Cutaneous ageing is a complex biological process affecting all skin components. It consists of two independent, clinically and biologically, distinct processes. The first is intrinsic or innate ageing, which affects the skin in the same pattern as it affects all internal organs. The second is extrinsic ageing, which is the result of exposure to external factors, mainly ultraviolet (UV) irradiation [1].

Extracellular matrix molecules are highly implicated in the ageing process and exhibit specific alterations in extrinsic and intrinsic skin ageing [2]. Among them, hyaluronic acid (HA) is of high importance since it has the unique capacity of binding water, thus providing viscosity and hydration to the dermis. In photo-ageing process, HA homeostasis shows specific alterations since in photo-aged skin HA of reduced size is elevated and exhibits abnormal deposition [3].

In this study, we have tried to elucidate alterations in HA homeostasis and proteoglycan expression associated with intrinsic skin ageing. We employed juvenile skin tissue specimens ($n = 10$, mean age 5 years, 4 mm punch biopsies) collected from foreskin of children undergoing surgery for phimosis. Adult photo-protected skin tissue specimens ($n = 16$, mean age 72 years, 4 mm punch biopsies) were collected from the area behind the ear lobe. Total glycosaminoglycans were isolated and purified from skin tissue specimens, as previously described [3]. Aliquots of total glycosaminoglycans were assayed for HA content by ELISA. Gene

expression of HYAL, HAS, CD44, RHAMM and proteoglycans was assessed by RT-PCR [3]. Statistical analyses were performed using SPSS 12.0 for Windows.

We report that the relative amount of HA in adult skin tissue specimens was significantly less (p-value: 0.0001) compared with juvenile skin (160 ± 19 versus 310 ± 27 ng/μg of uronic acids). Furthermore, gene expression of HAS-1, HAS-2, HYAL-2 and HYAL-3 was significantly down-regulated in adult photo-protected skin (Fig. 1), indicating lower HA metabolic rate in intrinsic skin ageing, resulting in decreased content of HA in the adult skin. This observation is in agreement with previous studies, employing tissue staining, which demonstrated that there is a progressive reduction in the number of electron-dense granules of HA and their filaments with age [4]. The reduced HA content could account for some of the most striking alterations of the aged skin, including decreased turgidity, less support for micro vessels, wrinkling and altered elasticity.

The biological effects of HA are mediated following binding of HA to its receptors CD44 and RHAMM. In the present study, we showed that gene expression of both receptors is decreased following intrinsic ageing (Fig. 1). This may reflect to reduced or abnormal function of HA in the aged skin. Furthermore, it has been shown that binding of HA to CD44 is a prerequisite step for HA degradation. Thus, the reduced expression of HAS, HYAL and CD44 that we report here, results to reduced turnover of HA and to extracellular deposition of lower amounts of HA which may be regarded as "aged" and probably less functional.

We have previously shown that extrinsic skin ageing is associated with alterations in the expression of HA and its metabolizing enzymes [3]. However, HA homeostasis in extrinsic skin ageing exhibits a different profile than in intrinsic skin ageing reported in this study. Extrinsic ageing is characterized by increased amounts of HA of lower molecular mass, reduced HAS and increased HYAL expression, whereas HA receptors are down-regulated [3]. These findings suggest that intrinsic and extrinsic skin ageing are two distinct biological processes, both associated with abnormal HA turnover.

In the present study, we further investigated the expression of various proteoglycans in intrinsic skin ageing, since these molecules have been shown to regulate the mechanichal properties of the skin, tissue hydration and resiliency. We found that with the exception of aggrecan which was significantly up-regulated in the aged skin, all other proteoglycans were significantly down-regulated (Fig. 2).

It has been shown that the mRNA level of aggrecan is up-regulated in skin fibroblasts of Hutchinson–Gilford Progeria Syndrome, a disorder of accelerated ageing which exhibits similar characteristics to intrinsic skin ageing [5]. The increased expres-

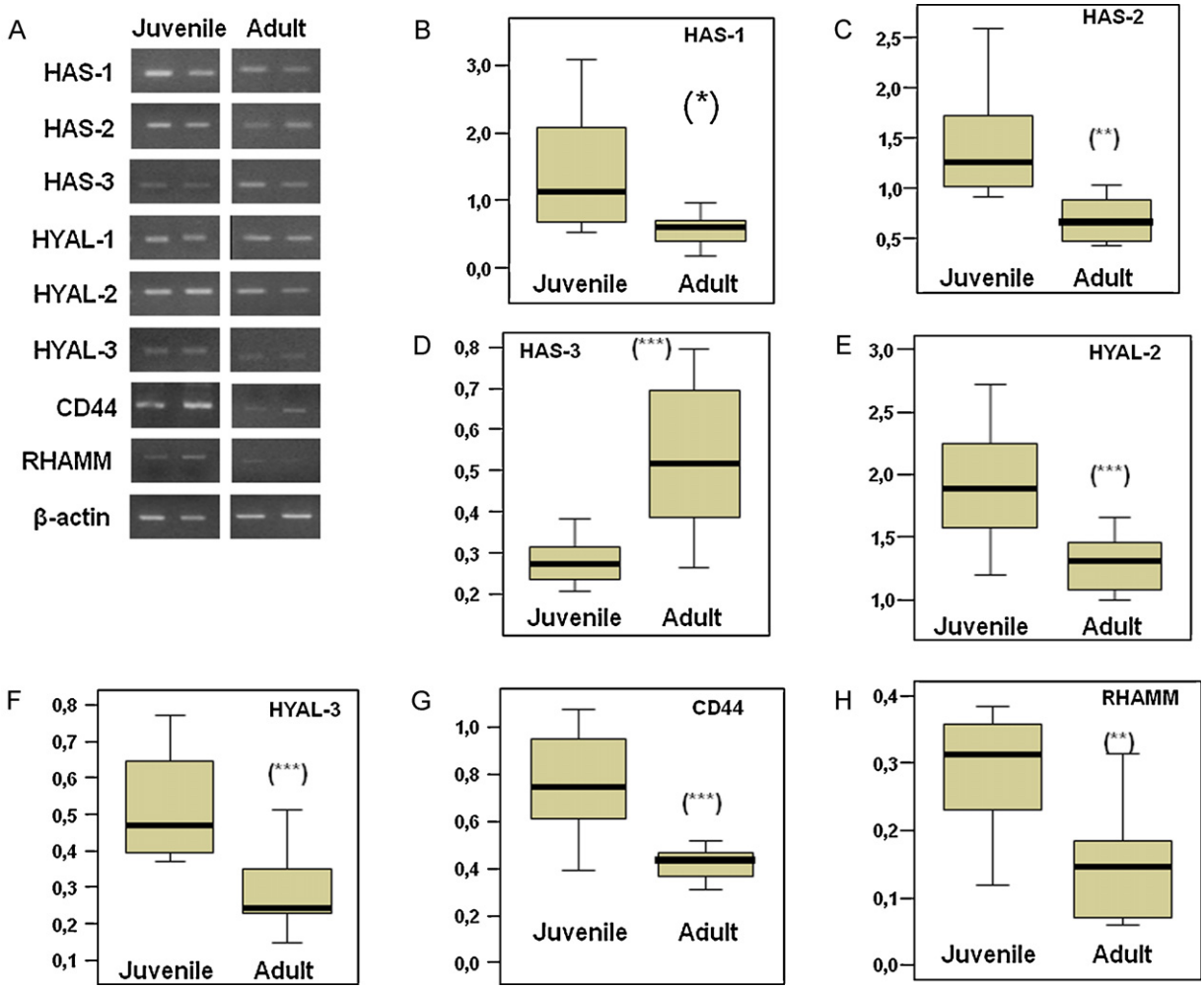

Fig. 1. Gene expression of HAS, HYAL, CD44 and RHAMM. (A) Representative RT-PCR analysis of HAS, HYAL, CD44 and RHAMM. Densitometric ratios of HAS-1/β-actin (B), HAS-2/β-actin (C), HAS-3/β-actin (D), HYAL-2/β-actin (E), HYAL-3/β-actin (F), CD44/β-actin (G), and RHAMM/β-actin (H). Data are presented as boxplot analysis of RT-PCR results obtained from juvenile (n = 10) and from adult (n = 16) photo-protected skin tissue specimens. The length of the box contains 50% of cases. The line across the inside of the box represents the median value. Lines protruding from the box go out to the variable's smallest and largest values. *p-Value between 0.05 and 0.01, **p-value between 0.01 and 0.005, ***p-value < 0.005.