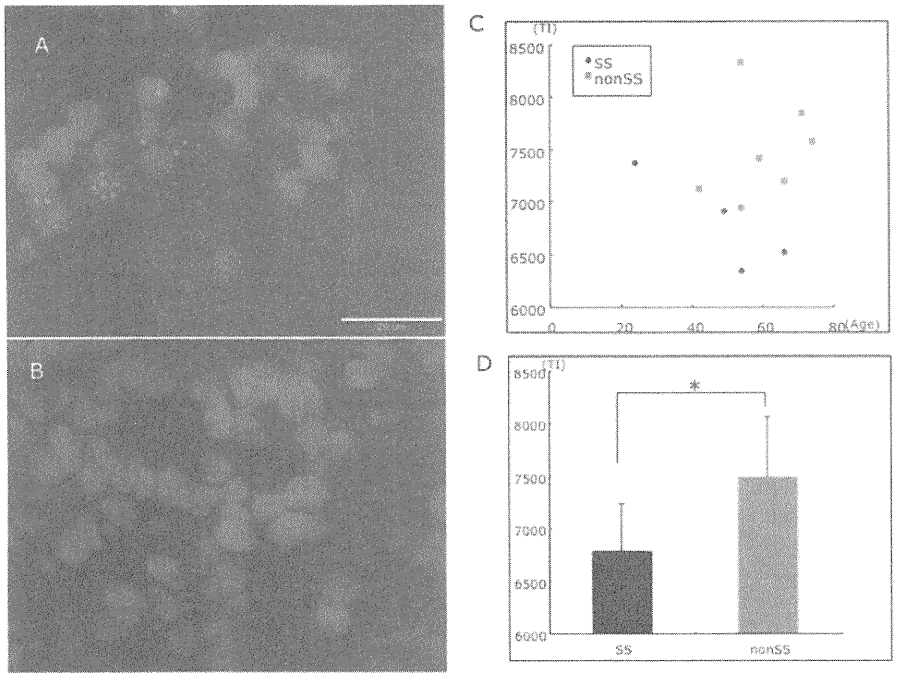

Figure 1. Telomere shortening in Sjögren syndrome. Representative telo-FISH photograph of non-Sjögren syndrome (A) and Sjögren syndrome (B) lacrimal glands. Scatter plot of age and telomere intensity (TI; C). Comparison of average telomere intensity in Sjögren and non-Sjögren syndrome groups demonstrated that telomeres were significantly shorter in Sjögren syndrome group (D; p=0.02).

markers weaker or non-existent in the Sjögren syndrome group.

Electron microscopy findings: To determine ultrastructural changes in lacrimal gland, we analyzed samples using electron microscopy. Electron microscopy revealed that the structure of each lacrimal acinar unit was compact and uniform in the non-Sjögren syndrome group. Myoepithelial cells were smooth in shape (Figure 3B). Mild acinar atrophy and fibrosis were observed more frequently in the Sjögren syndrome group (Figure 3E). Infiltration of inflammatory cells was observed in both groups, being particularly marked in the Sjögren syndrome group. High magnification revealed that the structure of mitochondrial cristae was severely damaged and swollen in the Sjögren syndrome group (Figure 3F) compared to that in the non-Sjögren syndrome group (Figure 3C), indicating that mitochondrial damage may be related to Sjögren syndrome.

DISCUSSION

In this study, we successfully measured telomere intensity in lacrimal gland epithelial cells by telo-FISH and investigated relative telomere length in each cell. We believe that this is the first report of a telomere length analysis in lacrimal gland.

The results showed that the telomeres in lacrimal gland cells in the Sjögren syndrome group were significantly shorter than those in the non-Sjögren syndrome group (p=0.02). Patchy invasion by inflammatory cells and the destruction of lacrimal gland structure were observed frequently in the Sjögren syndrome group. It should be noted that these results were obtained even though we selected only those areas in which acinar unit structure was preserved for telo-FISH. Furthermore, even though the clinical findings were similar between the two groups, telomere length showed a significant difference.

These results suggest that telomere length is related to severe dry eye diseases where normal lacrimal gland function has been disrupted by chronic inflammation. Recently, it has been reported that renal failure shortens cardiac telomeres, and that short telomeres are a risk factor for idiopathic pulmonary fibrosis [10,11]. The present results are consistent with these earlier reports indicating a strong association between organ dysfunction and telomere shortening, suggesting telomere shortening as a risk factor for lacrimal gland dysfunction as well. Telomere shortening has been reported in several inflammatory diseases such as vascular disease, type 2 diabetes, Fanconi anemia, and ataxia teleangiectasia [12-15]. Lacrimal gland epithelial cell turnover was not clearly defined until recently, however, so further study is necessary to investigate the relationship between telomere length and inflammation in this tissue.

Progenitor cell marker expression (p63, nucleostemin, and ABCG2) was weaker in the Sjögren syndrome group than in non-Sjögren syndrome group. p63 is often used as a progenitor cell marker for keratinocytes and corneal epithelial cells with high proliferative potential [20]. Nucleostemin has been reported in proliferating cells in various tissues, including bone marrow, and may be used as a progenitor marker for stratified epithelial cells [21,22]. ABCG2 and

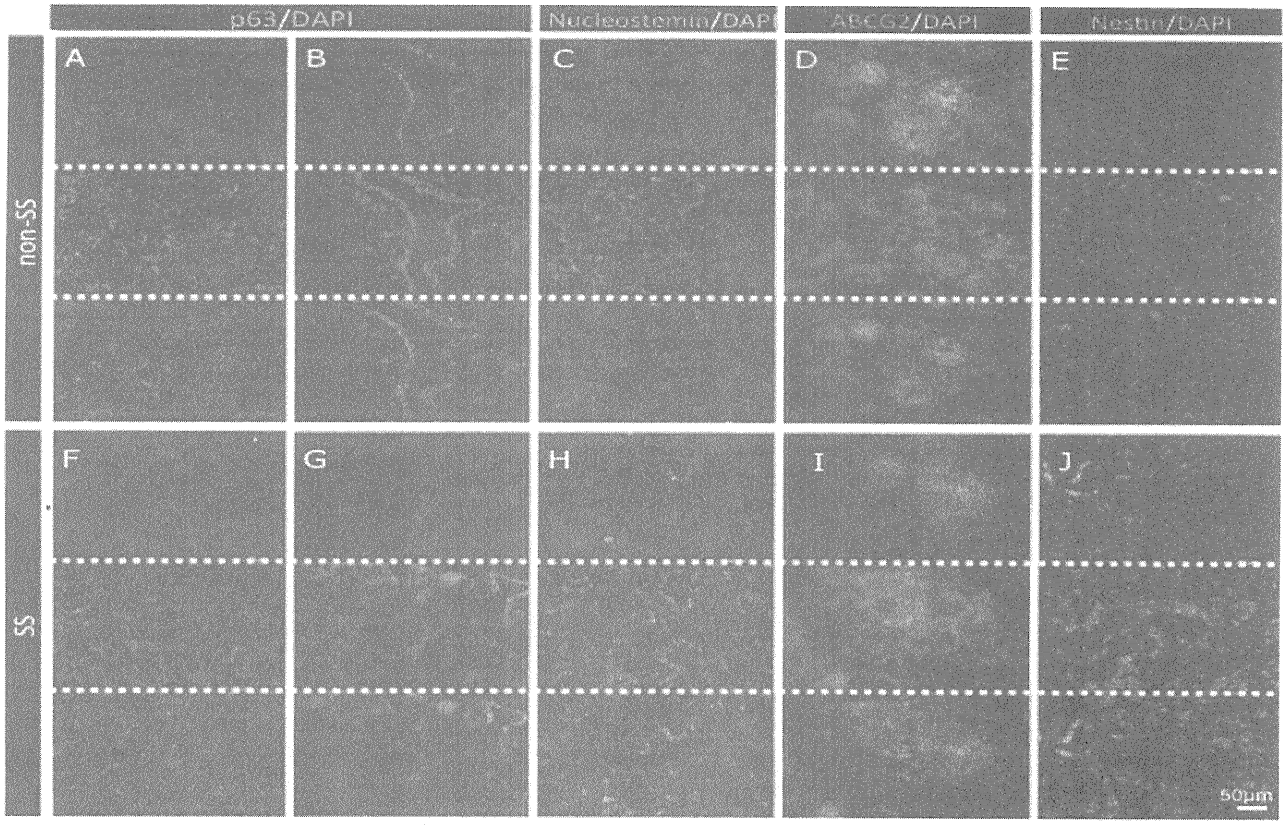

Figure 2. Immunostaining for progenitor markers. In non-Sjögren syndrome, p63 (red) was expressed in 2–4 cells in each acinar unit (**A**) and all ductal basal cells (**B**; case 7). In Sjögren syndrome, p63 was weakly expressed with irregular pattern. (case 2; **F**, **G**). Nucleostemin was expressed with a similar pattern in non-Sjögren syndrome (case 7; **C**) and Sjögren syndrome (case 2; **H**). Nuclei were counterstained with DAPI (blue). ABCG2 (red) was expressed in intercellular junction and cytoplasm in acinar unit (**F**, **I**), and weaker in Sjögren syndrome. Nestin was expressed strongly in some location in Sjögren syndrome (**E**, **J**). Scale bars indicate 50 μm (**A-C**, **E-H**, **J**) and 20 μm (**D**, **I**), SS=Sjögren syndrome, non-SS=non Sjögren syndrome.

nestin have been recognized as one of progenitor cell markers in adult tissue. Surprisingly, nestin-positve cells were observed more frequently in the Sjögren syndrome, which maybe partially explained that nestin expressed only in repairing/regeneration location, but not in quiescent cells [19,25]. These results suggest that telomere length shortening in lacrimal progenitor cells indicates the pathophysiological conditions necessary for development of Sjögren syndrome. Although most tissues are known to have their own tissue-specific stem cells, the existence of stem cells in lacrimal gland has yet to be proven. The results of telo-FISH may indicate the existence of progenitor cells in lacrimal gland [30]. Further investigation is needed to characterize the progenitor cells and their homeostasis in lacrimal gland.

Electron microscopy revealed that the structure of mitochondrial cristae was severely damaged in the Sjögren syndrome group (Figure 3F) compared to in the non-Sjögren syndrome group (Figure 3C). There were some reports about the associations among telomere length, mitochondrial function and oxidative stress [31-36]. Mitochondria are the most important source of reactive oxygen species in cells under physiologic conditions, and premature senescent cells sorted from young cultures displayed mitochondrial dysfunction, increased oxidative stress and short telomeres [31]. Another report showed that improvement in mitochondrial function results in less telomeric damage and slower telomere shortening, while telomere-dependent growth arrest is associated with increased mitochondrial dysfunction [32]. Furthermore, telomere-shortening rate and cell replicative life spans can be greatly modified by DNA-damaging oxidative stress [5], which has been shown to accelerate telomere shortening during DNA replication [37]. The mitochondrial structural changes observed in this study may contribute to the increase in oxidative stress induced by Sjögren syndrome. However the relationship between mitochondrial damage and telomere shortening was not clear in this study, and further study was necessary to clarify the molecular mechanism. The results of this study indicate that 1) telo-FISH is a viable method of assessing telomere length in lacrimal gland; and 2) telomere length in Sjögren syndrome

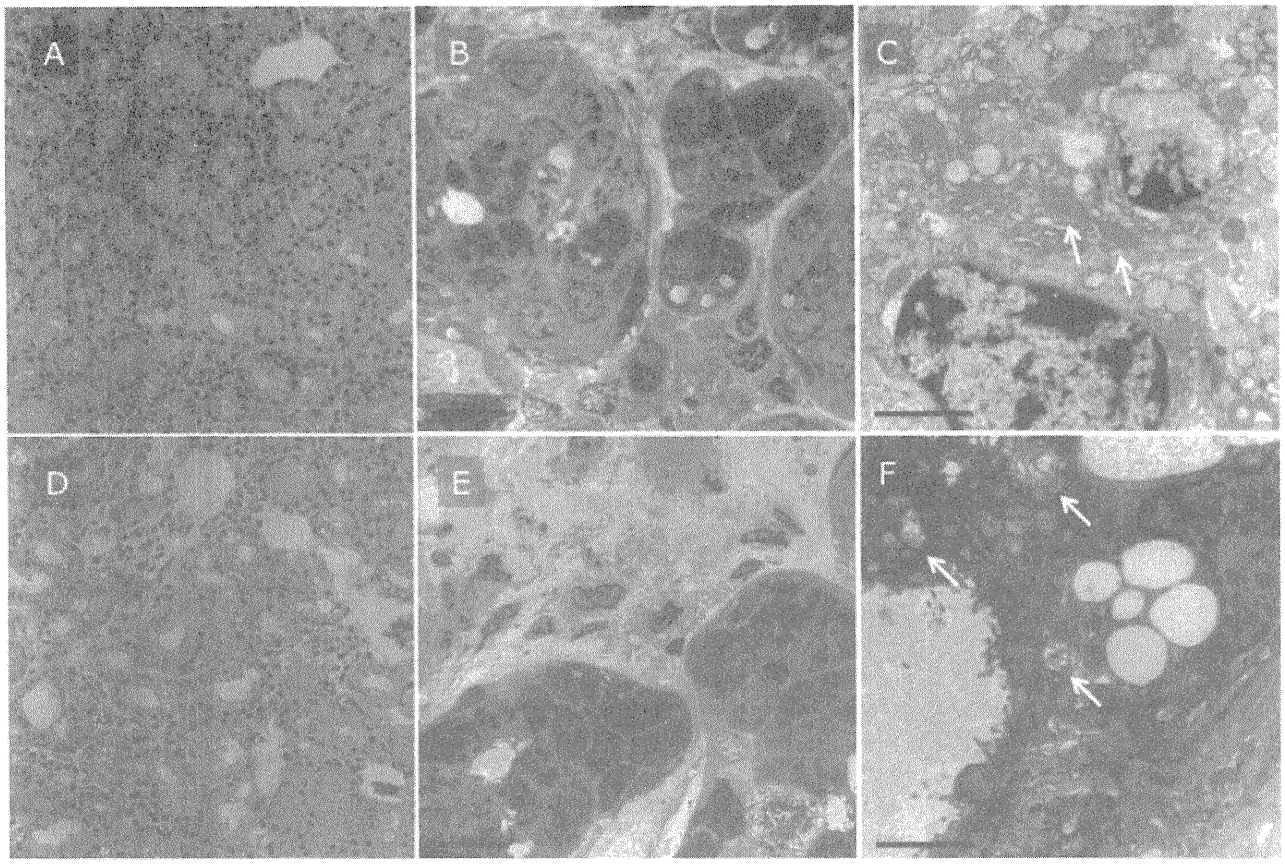

Figure 3. Electron microscopy of lacrimal gland. Representative H&E staining and electron microscopy photographs of non-Sjögren syndrome (case 9; **A**, **B**, **C**) and Sjögren syndrome patient (case 4; **D**, **E**, **F**) were shown. Scale bar indicated 5 μm (**B** and **E**) and 2 μm (**C** and **F**). Structure of lacrimal acinar unit was compact and uniform in non-Sjögren syndrome patients (**A** and **B**), but mild acinar atrophy and fibrosis were observed more frequently in Sjögren syndrome patients (**D** and **E**). High magnification revealed that structure of mitochondrial cristae (arrows) was severely damaged and swollen in Sjögren syndrome patient (**F**) compared to that in non-Sjögren syndrome patient (**C**).

is shorter than in non-Sjögren syndrome, possibly due to acceleration of the cell cycle to maintain lacrimal gland cell homeostasis, and associated with lower levels of expression of p63 and nucleostemin.

Taken together, this suggests that dysfunction in lacrimal gland may be related to epithelial cell telomere shortening. Further study is needed, however, to clarify the underlying molecular mechanism.

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