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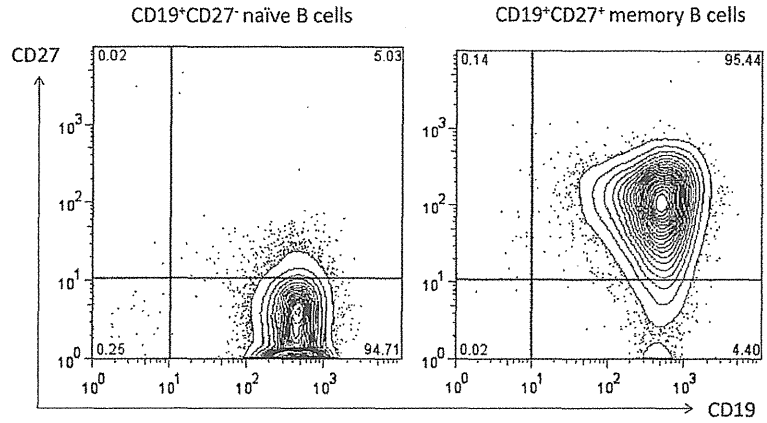

FIG E1. Phenotypic analysis of B-cell subsets in human peripheral blood. B cells were obtained by means of negative selection from PBMCs. $CD27^+$ memory B cells were then isolated by using positive selection from B cells with CD27 microbeads. The negative fraction of this isolation was assigned to $CD27^-$ naive B cells. The purity of naive and memory B cells was greater than 90% (*x-axis*, CD19; *y-axis*, CD27).

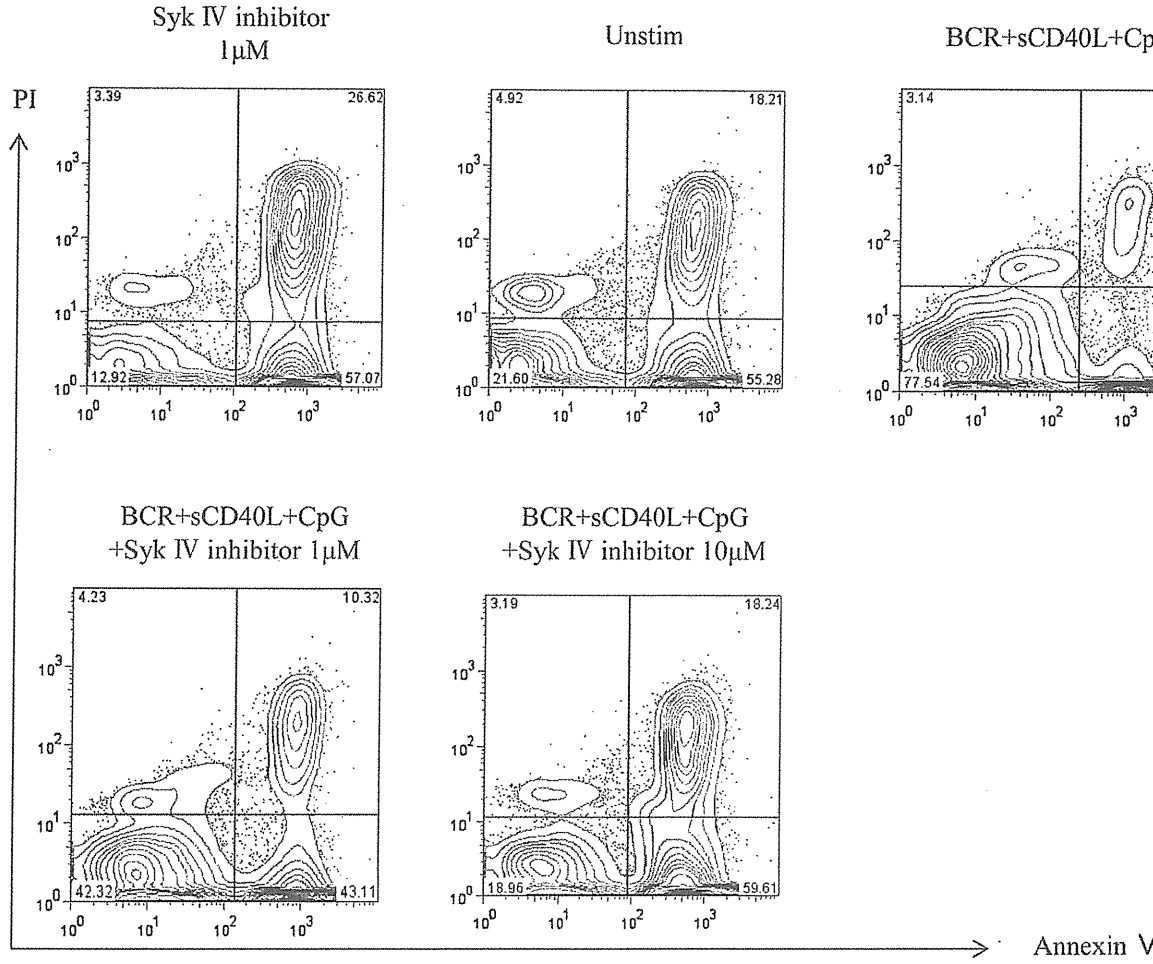

FIG E2. Syk provides survival signals for B cells after stimulation through all 3 receptors. B cells (2×10^5 per well) were cultured in triplicate in 96-well plates with anti-Igλ and anti-Igk antibodies (1 μg/mL), soluble CD40 ligand (*sCD40L*; 2 μg/mL), and CpG-ODN 2006 (2.5 μg/mL) with or without Syk inhibitor IV for 72 hours. The percentage of apoptotic B cells was assessed by means of double-staining with FITC–Annexin V and PI (*x-axis*, PI; *y-axis*, Annexin V).

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ORIGINAL ARTICLE

Prevalence and incidence of polymyositis and dermatomyositis in Japan

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Abstract

Objectives. To estimate the number of patients with polymyositis/dermatomyositis (PM/DM) in Japan and the prevalence rate and incidence rate of the disease.
Methods. The electronic database in the nationwide registration system on intractable diseases from 2003 to 2010 was utilized to identify the number of prevalent and incident cases of PM/DM. The electronic data entry rate was used to establish the total number of registered cases.
Results. The estimated total number of patients with PM/DM and the prevalence rate in Japan in 2010 were 17,000 and 13.2 per 100,000 population, respectively. The prevalence of PM/DM ranged from 10 to 13 per 100,000 population with a trend toward increasing over time. The incidence of PM/DM was estimated within the range 10–13 per 1,000,000 person-years, except for 2003.
Conclusions. We report the prevalence and incidence of PM/DM recently in Japan for the first time at the nationwide population level. Because the prevalence seems to be increasing recently, continued monitoring of these epidemiologic features is required.

Keywords

Epidemiology, Incidence, Nationwide survey, Polymyositis/dermatomyositis (PM/DM), Prevalence

History

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Introduction

Polymyositis (PM) and dermatomyositis (DM) are chronic idiopathic inflammatory disorders, affecting the skeletal muscles, the skin and other organs. They are rare, but their chronic intractable nature has a significant impact on the utilization of medical care resources, the patients' activities of daily living, and their quality of life. The epidemiologic features of PM/DM, such as prevalence and incidence, are not well documented. In order to understand the clinical and public health importance and to plan for disease control and prevention, it is essential to estimate incidence and prevalence rates, and to know the total number affected in the population.

As PM/DM are rare diseases, only limited epidemiologic studies have been undertaken, and mostly the incidence investigated in Western countries [1–9]. Few studies have been conducted in Asian populations [10, 11]. Prevalence data on PM/DM are even more scarce [4, 11–13]. PM/DM incidence and prevalence reported in the literature have been estimated only in relatively small populations, and are likely to have correspondingly large variance. For rare diseases, epidemiological observations in large populations are required for accuracy. The incidence of PM/DM in Japan has not been estimated to date in any nationwide survey. The prevalence of PM/DM in Japan was estimated from a nationwide survey in 1991 [13]. However, no other reports on prevalence in Japan have appeared since. It is therefore worthwhile to estimate the recent incidence and prevalence of PM/DM in Japan at the nationwide population level.

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The National Program on Rare and Intractable Diseases was launched by the government in Japan in 1972 to promote research on a number of rare and intractable diseases [14]. It increased support for patients by subsidizing their health care expenditure and provides a nationwide registration system for diseases including PM/DM, systemic lupus erythematous, systemic sclerosis (SSc) and some other autoimmune diseases. In the present study, we used this database to estimate the total numbers of patients with PM/DM in Japan, and the prevalence and incidence rates of the disease.

Materials and methods

Data sources

The database in a nationwide registration system established by the Japanese government for patients with intractable diseases including PM/DM was utilized for estimating prevalence and incidence. Patients with PM/DM desiring a subsidy for their medical care, must apply for aid. If the application is accepted, the recipient is recorded in the registry. The duration of the subsidy is 1 year, effective from October to September of the following year. Renewed application and re-registration in the system are required every year, if the patients wish to continue to receive financial support. The details of the registration system have been described elsewhere [14]. Diagnoses of patients with PM/DM were established according to the standard criteria ordained by the Ministry of Health, Labour and Welfare (MHLW) of Japan [15]. Approximately 40–80% of registered patients' data were converted into electronic form, which we utilized to collect the characteristics of each patient. This electronic database has been available for epidemiological research since 2003.With permission from the MHLW, we used the data from Japanese fiscal years 2003 to 2010.

The fiscal year means from April to March of the following year. The electronic converted data indicate the year of disease onset for each patient.

Statistical analysis

We calculated the electronic data entry rate as the number of patients whose data were converted into electronic form divided by the total number of patients enrolled in the registration system for each fiscal year from 2003 to 2010. The latter information is reported in MHLW's Report on Public Health Administration and Services [16]. As the number of registered patients with PM/DM is reported together with SSc in the report, we added the number of patients with PM/DM and SSc together in the electronic converted data to give the electronic data entry rate. Thus, the electronic data entry rate is the number of PM/DM and SSc patients whose data were converted into electronic form divided by the number of all registered patients with PM/DM and SSc. Because we could not extract the electronic data entry rate exclusively for PM/DM, we assume that the data entry rate of PM/DM is not different from that of PM/DM and SSc combined. We also assume that both the entry rate of new recipients and annual renewals are the same, and also that they are identical with the rate of all patients (new and renewals summed together) in each year.

The number of PM/DM cases in each year to estimate prevalence was the number of PM/DM patients whose data were converted into electronic form divided by the electronic data entry rate.

The number of PM/DM cases to calculate incidence was estimated as follows: for the number each year, we used the onset year of the electronic converted patients. The number of patients initially registered and converted into electronic form in fiscal year-j and onset at year-i, Nij, divided by the electronic data entry rate in fiscal year-j are summed for each year-i to yield the number of incidence cases in year-i as the following equation shows:

$$Ai = \sum_{j=i-1}^{2010} \frac{Nij}{Pj}$$

i = onset year (i = 2003, 2004,..., 2010)

j = initially registered fiscal year (j = 2003, 2004,..., 2010)

$i \leq j$

Ai: number of incidence cases in year-i

Nij: number of patients initially registered and converted into electronic form in fiscal year-j and onset at year-i

Pj: electronic data entry rate in fiscal year-j

The Japanese census population in 2005 and 2010 and the estimated population in the other years are used as the denominator of the prevalence rate and incidence rate. All statistical analyses were performed with SAS version 9.1.3 software (SAS Institute Inc., Cary, NC, USA).

Ethical considerations

All data provided by the MHLW are anonymous, and researchers cannot access personal information about any of the patients.

Results

Table 1 shows the electronic data entry rate of PM/DM and SSc, and estimated number of patients with PM/DM in fiscal years 2003–2010. The number of patients with PM/DM and SSc whose data were converted into electronic form ranged from 16,388 to 33,309 each year, with the highest number in 2009. Dividing by the total number of registered patients with PM/DM and SSc enrolled in the registration system which was obtained from the MHLW's Report on Public Health Administration and Services, the electronic data entry rates were obtained. They ranged from 39% to 80%, highest in 2009. The number of registered patients with PM/DM whose data were converted into electronic form ranged from 6,328 to 13,710 which was divided by the entry rate to yield the prevalent number of patients each year. The estimated number of PM/DM patients in 2010 was 17,000, ranging in each year from 2003 to 2010 from 13,000 to 17,000, thus tending to increase over the years. These numbers can be considered to represent the total number of registered patients in the whole of Japan (population 127 million). The estimated prevalence of PM/DM in Japan in 2010 was thus 13.2 per 100,000 population. The prevalence of PM/DM ranged from 10 to 13 per 100,000 population over the years 2003–2010, increasing over time. Thus, prevalence increased 1.3-fold during this time.

The incidence of PM/DM in each year from 2003 to 2010 was estimated from the number of cases initially registered in that year, and converted into electronic form. The figures in Table 2 show the number of patients initially registered and electronically converted, excluding the cases with disease prior to 2003. The number of cases in each year is separated according to their year of onset and divided by the electronic data entry rate of that registration year to yield the estimated number of incidence cases in each year from 2003 to 2010. This ranged from 900 to 1,700 per year (Table 3). The incidence rate of PM/DM in Japan was estimated as ranging from 10 to 13 per 1,000,000 person-years (except for

Table 1. Electronic data entry rate for PM/DM and SSc (on June 2011), and estimated prevalence of PM/DM in fiscal years 2003–2010.

	PM/DM and SSc			Estimation of prevalence of PM/DM		
Fiscal year	No. of electronic entries of PM/DM and SSc patients[a]	Total No. of registered patients with PM/DM and SSc[b]	Electronic data entry rate[c]	No. of electronic entries of PM/DM patients[d]	Estimated number of patients with PM/DM[e]	Estimated prevalence (per 100,000 population)[f]
2003	20,162	31,829	0.633	8,332	13,163	10.3
2004	21,709	32,944	0.659	9,043	13,722	10.7
2005	22,057	34,592	0.638	9,327	14,619	11.4
2006	20,031	36,110	0.555	8,139	14,665	11.5
2007	16,388	37,975	0.432	6,328	14,648	11.5
2008	20,242	39,970	0.506	7,919	15,650	12.3
2009	33,309	41,648	0.800	13,710	17,138	13.4
2010	16,528	42,233	0.391	6,618	16,926	13.2

PM/DM, polymyositis/dermatomyositis; SSc, systemic sclerosis.

[a]The number of patients with PM/DM and SSc whose data were converted into electronic form.

[b]Total numbers of registered patients with PM/DM and SSc enrolled in the registration system, obtained from the Report on Public Health Administration and Services [16].

[c]a/b.

[d]The number of patients with PM/DM whose data were converted into electronic form.

[e]d/c.

[f]The Japanese census population in 2005 and 2010 and estimated population in the other years are used as the denominator of the prevalence rate.

Table 2. Number of initially registered patients with PM/DM whose data were converted into electronic form and whose year of onset year was 2003–2010.

Initially registered fiscal year	No. of patients
2003	338
2004	668
2005	921
2006	683
2007	715
2008	956
2009	1,240
2010	861
Total	6,382

PM/DM, polymyositis/dermatomyositis.

2003). The incidence rates in 2009 and 2010 are lower than in the other years.

Discussion

In this study, we estimated the number of patients with PM/DM in Japan, and also calculated prevalence and incidence of the disease based on the nationwide survey. We estimated the number of patients currently affected (e.g. prevalent cases) in Japan and its prevalence per 100,000 population. We also estimated the incidence of the disease in Japan at the nationwide population level for the first time. These results provide basic information for disease control and prevention and planning public health policy.

PM/DM are rare diseases and earlier reports on incidence and prevalence are limited. The reported incidence of PM/DM ranges from 2 to 10 per 1,000,000 person-years in different populations between the 1940s and the 1990s [1–11]. Furthermore, there is a trend toward increasing incidence over time also in these studies. Earlier prevalence data are also very limited. Estimates of prevalence from the USA [4] and Japan [11–13] range between 2.4 and 9.9 per 100,000 population. Different diagnostic and classification criteria were employed in these studies, partly explaining the diverse reported incidence and prevalence rates in these studies. Some reports determined incidence from retrospective hospital-based studies in which the true incidence of PM/DM may have been underestimated. Furthermore, as the number of patients included in most of these earlier studies was small, only 40–100, the estimates may be relatively unreliable. The increasing incidence over time may be due to an increased physician awareness of the disease, progress in diagnostic techniques or increased availability of tests and better medical records, but could also reflect a true increase in disease occurrence.

The estimated prevalence per 100,000 population of PM/DM in Japan was 10–13 in 2003–2010, tending to increase over the years. The incidence of PM/DM per 1,000,000 person-years was estimated as 10–13, except in 2003. The prevalence and incidence of PM/DM in our study are higher than in most previously reported estimates. These differences may be due to the lack of standardization of the diagnostic criteria employed, as well as different ethnicities of the patients. Easier access to medical care via the public insurance system and financial support program in Japan may be one more reason for the higher estimates in this country. Cases in this study were identified by the standard diagnostic criteria ordained by the MHLW of Japan [15]. The significance of our study depends on the clearly defined standard criteria and large scale population review. The estimated incidence is based on a large number of approximately 6,400 patients during 8 years (Table 2).

A comparable total number of PM/DM patients in Japan are also available from a nationwide survey which accumulated number of patients reported from random-sampled hospitals from all over Japan, although the methodology of that survey was different from our own. The total number of patients with PM/DM in Japan was estimated at 3,000 (95% confidence interval 2,800–3,300) for PM and the same number (95% confidence interval 2,800–3,200) for DM, in 1991 [13]. Our estimate for PM/DM in Japan was 17,000 in 2010. This implies an increase of 2.8-fold (17,000/6,000) between 1991 and 2010. Our study also determined an increase in PM/DM prevalence over time. In addition to the above-mentioned factors which manifest as apparent increases in incidence rate, such as the use of different diagnostic criteria and physician awareness of the disease, better prognosis as result of improved treatment might also account for this trend.

Our estimated incidence values in 2009 and 2010 are slightly lower than 2008. This could be an underestimate because cases which would be registered from 2011 onward were not included.

There are some limitations to this study. First, there may be a possible bias due to the use of data from the Japanese government's registration system. Regarding the accuracy of the data we used, some degree of over- and/or underdiagnosis may exist, although specialist committees organized in each prefectural government check the diagnoses according to the standard criteria ordained by the government. With respect to coverage of the patients, most are expected to be diagnosed and registered in this system, but clearly we cannot be sure of the number of omissions. Patients who do not need financial support, some pediatric patients and patients whose medical expenses are covered by local government in an alternative financial support system will not apply to the system we used and thus be missing from the database. Thus, our study may underestimate prevalence. Second, we could not distinguish between PM and DM prevalence and incidence separately. This is because the national registration system combines these two diseases for administrative reasons, even though they are subgrouped

Table 3. Estimated number of incidence cases and incidence rate of PM/DM by onset year.

	Initially registered fiscal year									
Onset year	2003	2004	2005	2006	2007	2008	2009	2010	Total[a]	Incidence per 1,000,000 person-years[b]
2003	466	209	69	40	23	32	23	3	865	6.8
2004	68	750	260	63	60	26	21	15	1,263	9.9
2005		55	1,016	204	63	61	25	49	1,473	11.5
2006			99	886	301	99	46	46	1,477	11.6
2007				38	1,146	356	78	61	1,679	13.1
2008					63	1,253	290	110	1,716	13.4
2009						63	1,026	442	1,531	12.0
2010							41	1,476	1,517	11.8

PM/DM, polymyositis/dermatomyositis.

[a]Estimated number of incidence cases.

[b]The Japanese census population in 2005 and 2010 and estimated population in the other years are used as the denominator of the incidence rate.

based on distinct clinical and pathologic features. If we use the information of the skin manifestation of all the patients, PM and DM could be separated. Although the skin information of some proportion of patients is not well described in the database, further consideration may solve the problem in further study.

In conclusion, we provide estimates of the recent prevalence of PM/DM, and the incidence of the disease in Japan for the first time at the nationwide population level. As prevalence has been showing an increasing trend recently, a continuing concern must be to maintain monitoring of these epidemiologic features.

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Conflict of interest

H. Kohsaka has served as a consultant to Chugai Pharmaceutical and has received research grants from Eisai Pharmaceutical and Takeda Pharamaceutical. All other authors have declared no conflicts of interest.

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ORIGINAL ARTICLE

Age at onset and gender distribution of systemic lupus erythematosus, polymyositis/dermatomyositis, and systemic sclerosis in Japan

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Abstract
Objectives The aim of this study was to describe age, gender distribution, and age at onset of systemic lupus erythematosus (SLE), polymyositis/dermatomyositis (PM/DM), and systemic sclerosis (SSc) in Japan.
Methods We used epidemiological information on 21,405, 6,327, and 10,058 patients with SLE, PM/DM, and SSc, respectively, in a Japanese nationwide registration database of patients with intractable diseases.
Results All three diseases occur predominantly in women, with the female-to-male ratio being 8.2:1, 2.6:1, and 7.7:1 for SLE, PM/DM, and SSc, respectively. The most susceptible age for SLE is 15–44 and 20–39 years for males and females, respectively. For PM/DM it is 45–64 and 40–64 years and for SSc, 50–69 and 40–59 for men and women, respectively.
Conclusions The basic descriptive epidemiological characteristics of SLE, PM/DM, and SSc in Japan, such as gender distribution, present age, and age at onset, were surveyed nationwide for fiscal 2007. It was found that these characteristics were similar to those in Western populations. Our finding provides new information on the natural history of disease development.

Keywords Age at onset · Epidemiology · Polymyositis/dermatomyositis (PM/DM) · Systemic lupus erythematosus (SLE) · Systemic sclerosis (SSc)

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Introduction

Systemic lupus erythematosus (SLE), polymyositis/dermatomyositis (PM/DM), and systemic sclerosis (SSc) are systemic autoimmune diseases. Their chronic intractable nature has a significant impact on medical care utilization, activity of daily living, and quality of life. As these diseases are relatively rare, their epidemiological characteristics have not yet been described in detail in Japan. In such rare diseases, accumulation of large numbers of patients from the entire country is necessary for informative epidemiological studies.

The National Programme on Rare and Intractable Diseases was launched in Japan in 1972. Since then, the government has promoted research and expanded support for patients with a number of such diseases [1]. This programme established a nationwide registration system for patients with intractable diseases, including SLE, PM/DM, and SSc. Here, we describe age, gender distribution, and age at onset of these diseases using data from the registration system.

Materials and methods

Data sources

The Japanese government has established a nationwide registration system for patients with intractable diseases

under which registered patients are eligible for financial aid from the government for their treatment. Details of the registration system have been described elsewhere [1]. Most patients with SLE, PM/DM, or SSc are expected to be registered as having a designated intractable disease, although not all registered patients' data have been converted to electronic form. The electronic database has been effectively utilizable for epidemiological research since 2003. After obtaining permission from the Ministry of Health, Labour and Welfare (MHLW) of Japan, we used data of fiscal year 2007 consisting of patient sex, age, birth year, and disease-onset year.

Statistical analysis

We calculated the electronic data entry rate as the number of patients whose data was converted into electronic form divided by the total number of patients enrolled in the registration system. The latter information is contained in MHLW's Report on Public Health Administration and Services [2]. We ascertained age at disease onset as onset year minus birth year. Using onset age, we determined the most susceptible age as the minimum range that includes peak onset age and 50 % of onsets. All statistical analyses were performed with SAS version 9.1.3 software (SAS Institute Inc., Cary, NC, USA).

Ethical considerations

All data provided by the MHLW are anonymous, and researchers cannot access personal information about any patient.

Results

Table 1 shows the number of patients with SLE, PM/DM, and SSc whose data was converted into electronic form and the electronic data entry rate in fiscal 2007. We used electronic data for 21,405, 6,327, and 10,058 SLE, PM/DM, and SSc cases, respectively. The numbers of all patients registered with the database for the MHLW's Report on Public Health Administration and Services were 55,021 SLE and 37,975 PM/DM and SSc (PM/DM and SSc were not reported separately) [2]. The proportion of all patients with electronic data entered was therefore 39 % for SLE and 43 % for PM/DM and SSc. Estimating the number of all PM/DM- and SSc-registered patients separately, the number would be 14,714 (6,327/0.43) PM/DM and 23,391 (10,058/0.43) SSc. These can be considered to be the total number of registered patients in the entire Japanese (population 126 million).

Table 1 Number of patients with SLE, PM/DM, and SSc whose data was converted into electronic form, and the electronic data entry rate in fiscal 2007

Diseases	No. of electronic entries[a]	Total No. of patients[b]	Electronic data entry rate (a/b)
SLE	21,405	55,021	0.39
PM/DM	6,327	37,975	0.43
SSc	10,058		

SLE systemic lupus erythematosus, *PM/DM* polymyositis/dermatomyositis, *SSc* systemic sclerosis

[a] The number of patients whose data was converted into electronic form

[b] Total numbers of patients enrolled in the registration system was obtained from the 2007 Report on Public Health Administration and Services [2]

Table 2 Number of male and female patients with SLE, PM/DM, and SSc in fiscal 2007

Diseases	Total	Male	Female	Sex ratio
SLE	21,405	2,336	19,069	8.2
PM/DM	6,327	1,735	4,592	2.6
SSc	10,058	1,157	8,901	7.7

Sex ratio (female/male)

SLE systemic lupus erythematosus, *PM/DM* polymyositis/dermatomyositis, *SSc* systemic sclerosis

Table 2 shows the number of patients with SLE, PM/DM, and SSc stratified by sex. All three diseases predominantly affect women, with a female-to-male ratio of 8.2:1, 2.6:1, and 7.7:1 for SLE, PM/DM, and SSc, respectively.

Figure 1 shows the age distribution of male and female SLE, PM/DM, and SSc patients. The prevalence of SLE in women showed two peaks, at age 35–39 and 55–59 years, with a wide age distribution. The distribution of PM/DM was similar to SSc, with only a small number of patients <50 years, and peak prevalence at 55–59 years for PM/DM and 65–69 years for SSc. SLE distribution in men showed no significant age peak, and PM/DM and SSc were similar to that seen in women.

Figure 2 shows the distribution of age of onset. SLE onset peaked at 25–29 years in women, decreasing thereafter. Onset of both PM/DM and SSc in women also had one peak, but later, at 50–54 years of age, with PM/DM tending to have a younger onset than SSc. Age of SLE onset showed no peak in men, and again, PM/DM and SSc was similar in men and women.

These data on age at onset are summarized in Table 3. For SLE patients, age at the 50th percentile was 35 and

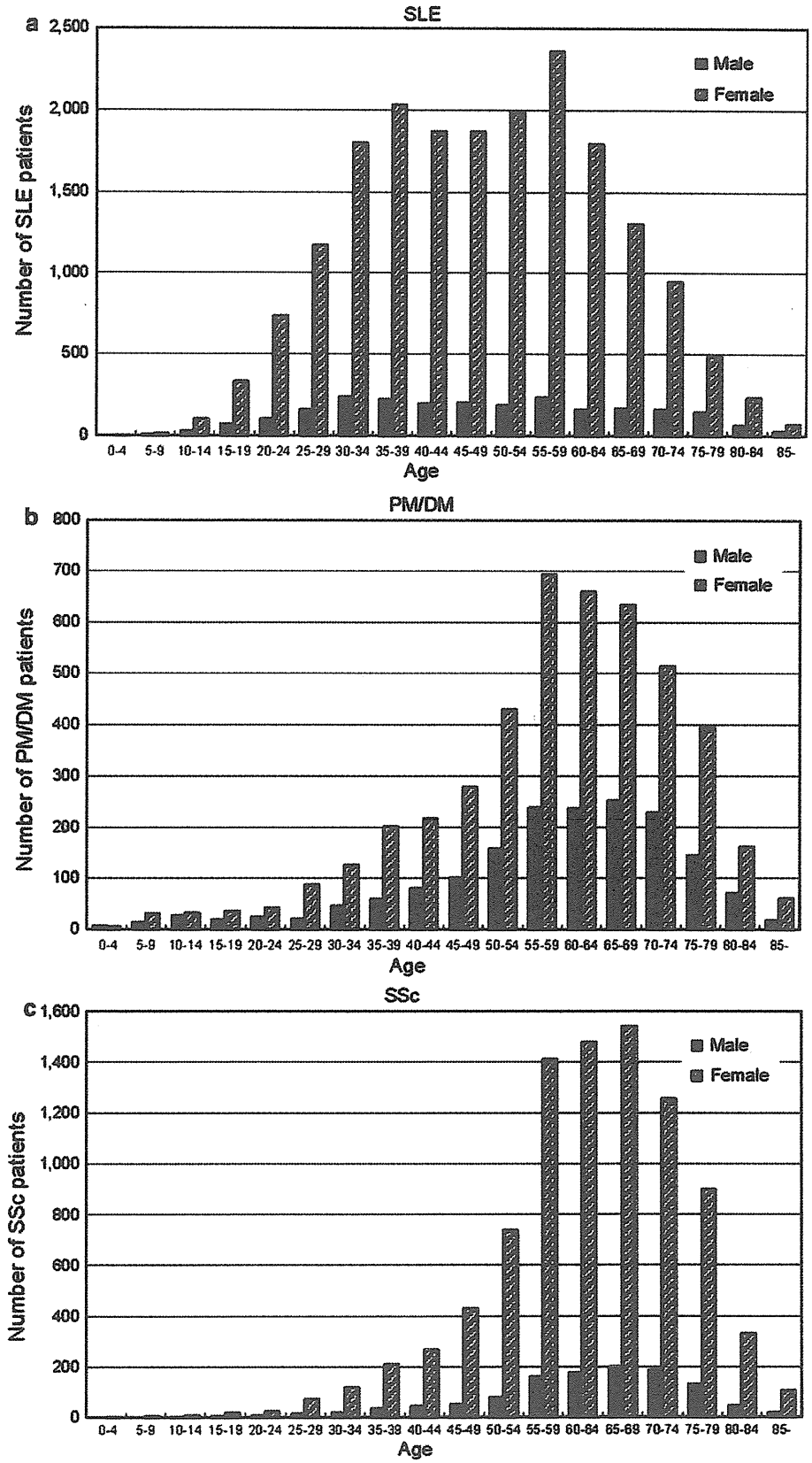

Fig. 1 Age distribution of SLE, PM/DM, and SSc in fiscal 2007. **a** *SLE* systemic lupus erythematosus, **b** *PM/DM* polymyositis/dermatomyositis, **c** *SSc* systemic sclerosis. *Solid bars* show the number of male patients, and *bars* with *right-slanting lines* show the number of female patients, by age

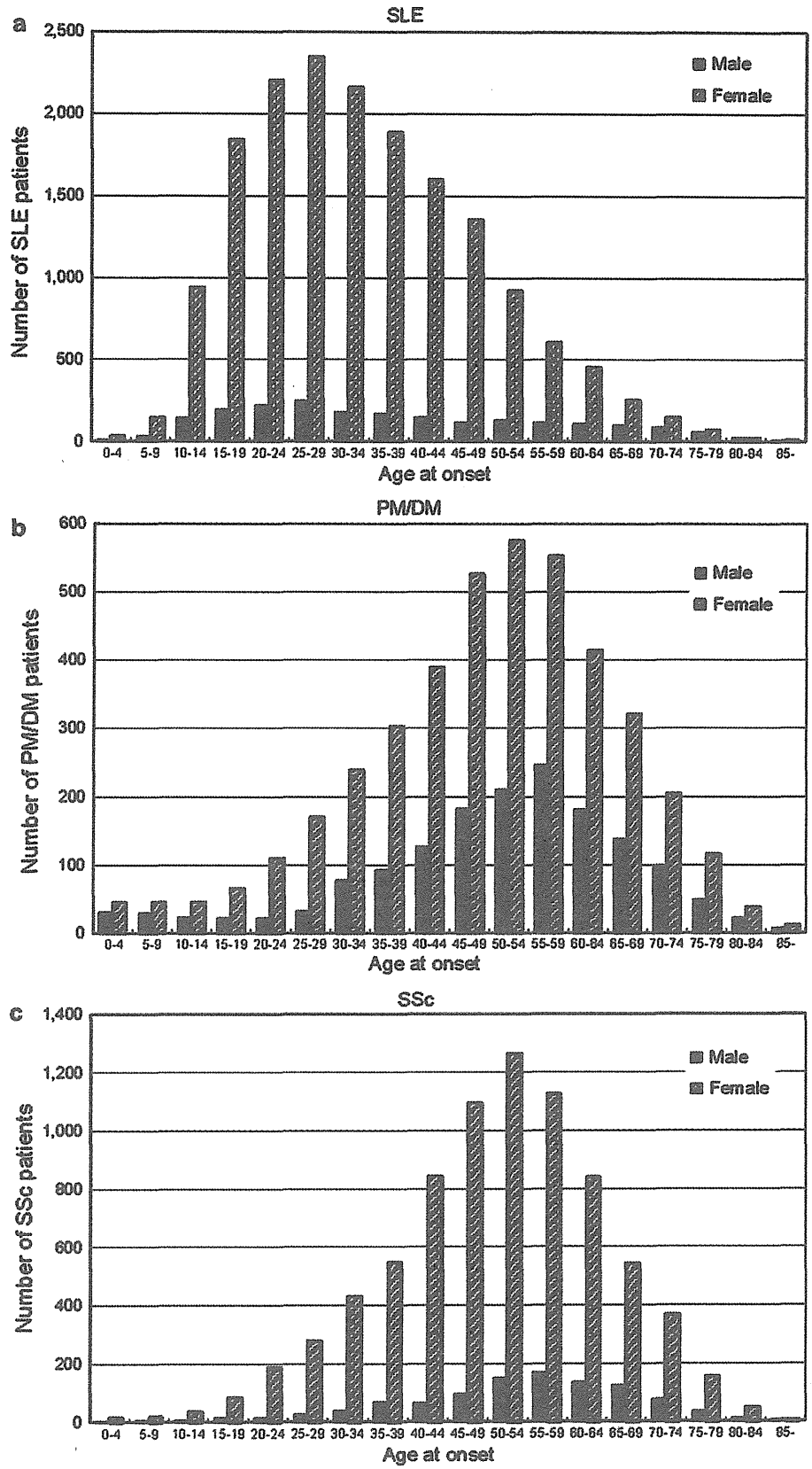

Fig. 2 Distribution of age at onset of SLE, PM/DM, and SSc in fiscal 2007. **a** *SLE* systemic lupus erythematosus, **b** *PM/DM* polymyositis/dermatomyositis, **c** *SSc* systemic sclerosis. *Solid bars* show the number of male patients, and *bars* with *right-slanting lines* show the number of female patients, by age at onset

Table 3 Distribution of age at onset of SLE, PM/DM, and SSc in fiscal 2007: percentile, mean, most susceptible age

Diseases	Sex	Number	Percentile (%)					Mean	SD	Most susceptible age[a] (years)
			10	25	50	75	90			
SLE	Male	2,042	15	23	35	54	68	38.5	19.3	15–44
	Female	16,995	16	22	32	43	54	33.7	14.5	20–39
PM/DM	Male	1,581	30	42	53	62	70	50.8	16.9	45–64
	Female	4,167	28	40	51	60	69	49.2	16.0	40–64
SSc	Male	1,018	35	46	55	64	70	54.0	14.1	50–69
	Female	7,875	32	42	51	59	67	50.2	13.7	40–59

Patients with unknown age at onset were excluded

SLE systemic lupus erythematosus, *PM/DM* polymyositis/dermatomyositis, *SSc* systemic sclerosis, *SD* standard deviation

[a] We determined the most susceptible age as the minimum range that includes the peak onset age and 50 % of onsets

32 years for men and women, respectively. For PM/DM and SSc, this was in the 50s for both sexes. Mean age at onset was similar to these 50th percentiles in all three diseases. We determined the most susceptible age as the minimum range that included peak occurrence and 50 % of onsets. This showed that men and women are most susceptible to SLE over the age range of 15–44 and 20–39 years, respectively. For PM/DM, this was 45–64 and 40–64, and for SSc, 50–69 and 40–59 years, for men and women, respectively.

Discussion

Here we report in detail present age and distribution of age at onset in patients with SLE, PM/DM, and SSc in Japan. Because these diseases are relatively rare, to produce an epidemiologically effective study, it is necessary to accumulate a large number of patients using nationwide surveys. We used data from a large number of such patients in Japan, which were especially informative regarding age of onset distribution. These data represent very important epidemiological information that has not been reported in any previous studies in Japan, with one exception that analyzed older data [3].

There are several reports describing age, occurrence frequency in men and women, and age of onset of SLE [4–6], PM/DM [7, 8], and SSc [9–11] in Western populations. In Caucasians, peak incidence of SLE occurs between ages 15 and 45 years, with a female-to-male ratio of 6–10:1 [4–6]. The pattern of occurrence of idiopathic inflammatory myopathy was bimodal, with a small childhood peak between 10 and 15 years and adult peak between 45 and 60 years [7, 8], with a female-to-male incidence ratio of 2.5:1 [7]. Age at onset of SSc is most commonly in the range of 45–65 years. As with the other two diseases, SSc is also predominant in women, with a female-to-male ratio of 4–6:1 [9–11]. The report presented here shows that the Japanese population seems similar to Western populations in the factors assessed here. However, age at PM/DM onset did not show a childhood peak. The reason for this may be that alternative financial support for medical treatment for children is provided by local government and therefore some of them are not registered in the national database. It means that a childhood peak may exist in Japanese, but our observation on the national database could not detect it.

There are some limitations to this study. One issue is data representativeness. The data we used, however, are derived from less than half of all registered patients, which depended on the electronic data entry rate. As the reason for the low entry rate is that some prefectures did not enter electronic data at a high enough rate due to financial or clerical problems, it is unlikely that the data entry rate differs between sexes and at different ages. In the observation of the characteristics of sex, age, and age of onset, we can expect that data is representative of the entire registered patient population. Other issues would be possible bias due to the use of data from the registration system, such as accuracy of disease diagnosis and coverage of all patients. Accuracy of data contained within this registration system is likely to be good, because specialist committees were organized in each prefectural government to check diagnoses according to standard criteria ordained by the government. Most patients are expected to be diagnosed and registered in this system, but clearly, we cannot be sure of the rate of omissions. Thus, calculations of prevalence rates from these data may be an underestimation. The prevalence of childhood patients may be also underestimated, as stated above. The last issue is the observation of incidence. We can distinguish newly registered cases, but as some of those may be recurrences or reregistration of patients who have moved (because when a patient changes address across prefectures, the registration may be renewed), not all the apparently newly registered cases are new incidence cases. Therefore, we did not show the incidence here.

In conclusion, gender distribution, present age, and age at onset of recent SLE, PM/DM, and SSc patients in Japan was surveyed nationwide for fiscal 2007. Our findings provide new information on the natural history of disease development in Japan, which, despite ethnic and other differences, appears similar to that familiar in Western populations.

Acknowledgments This study was supported by grants from Health and Labour Science Research of the Ministry of Health, Labour and Welfare, Japan: Research Committee on Epidemiology of Intractable Diseases and Research Committee on Autoimmune Diseases.

Conflict of interest None.

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Down-regulation of miR-223 contributes to the formation of Gottron's papules in dermatomyositis via the induction of PKCε

Background: Dermatomyositis (DM) is characterized by skin manifestations accompanying and preceding muscle weakness. Gottron's papules, one of the skin manifestations, are of great diagnostic value because they are specific to DM. However, the pathogenesis of Gottron's papules remains unclear. *Objectives:* We investigated the expression pattern of miRNAs in Gottron's papules of DM patients and evaluated the possibility that miRNAs play a role in its pathogenesis. *Materials and methods:* miRNAs were extracted from skin tissues and sera of patients with DM, clinically amyopathic DM (CADM) and healthy controls. To identify pathogenic miRNAs, we performed miRNA PCR array analysis. The results were confirmed by *in situ* hybridization, immunohistochemistry, immunoblotting and transient transfection of siRNAs or miRNA inhibitors. *Results:* PCR array analysis using tissue miRNAs demonstrated the miR-223 level was markedly decreased in Gottron's papules of DM and CADM *in vivo*, but not in psoriasis skin. The protein expression of PKCε, a predicted target of miR-223, was increased in DM/CADM skin. The transfection of a specific inhibitor of miR-223 in keratinocytes led to up-regulation of the PKCε protein, and resulted in increased cell proliferation. On the other hand, cell numbers were significantly decreased when cells were transfected with siRNA for PKCε. The serum miR-223 concentration was decreased in DM/PM patients, particularly in CADM patients, compared with healthy controls. *Conclusions:* A decreased miR-223 expression and the subsequently increased PKCε levels may therefore play a key role in the pathogenesis of Gottron's papules.

Key words: microRNA, autoimmune disease, collagen disease, keratinocyte, serum

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MicroRNAs (miRNAs) are small endogenous RNA molecules, about 22-25 nucleotides in length, which can regulate gene expression post-transcriptionally [1]. More than 1,000 miRNAs have been so far identified in humans [1, 2]. Thus, miRNAs are thought to be the most abundant class of regulators, and have been implicated in various cellular activities, including the immune response, cell development, cell differentiation, cell growth control and apoptosis.

The miRNAs are thought to be involved in the pathogenesis of various autoimmune diseases. For example, miR-146, miR-155 and miR-124 are reported to be associated with rheumatoid arthritis, while miR-574 and miR-768-3p are associated with Sjögren's syndrome. Similarly, miR-196a, miR-17-5p and miR-146 may contribute to the etiology of SLE, whereas miR-29a and miR-206 may play a role in systemic sclerosis [3-5]. However, little is known about the role of miRNAs in dermatomyositis (DM). Eisenberg *et al.* found several miRNAs that were dysregulated in the muscle tissues of DM patients [6]. However, the role of miRNAs in the cutaneous lesions of DM still remains poorly understood.

DM is diagnosed based on characteristic skin manifestations accompanying and preceding muscle weakness [7-10]. DM patients with clinically and histopathologically typical cutaneous lesions, but without myositis, are diagnosed as clinically amyopathic DM (CADM). The skin manifestations of DM and CADM include Gottron's papules, heliotrope rash, V-neck sign or shawl sign. The histopathological features of these eruptions include basal layer liquefaction degeneration, mild lymphocyte infiltration or dermal mucin deposition. Among the skin manifestations, Gottron's papules have diagnostic value because they are frequently specific to DM. Acanthosis, papillomatosis and hyperkeratosis as well as the basal layer liquefaction, inflammation and mucin deposition are distinctive features seen in Gottron's papules [11]. However, the pathogenesis of Gottron's papules, especially the cause

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of acanthosis or hyperkeratosis, remains unclear. In this study, we examined the expression pattern of miRNAs in the Gottron's papules of DM patients, and evaluated the possibility that miRNAs play a role in the pathogenesis of the disease.

Materials and methods

Patient material

Skin specimens were obtained from the Gottron's papules of 6 DM patients and 5 CADM patients. All DM patients fulfilled the criteria proposed by Bohan and Peter [7, 8]. The patients with CADM were diagnosed according to the previously described criteria [17]. Control skin samples were obtained from routinely discarded skin of healthy human subjects undergoing skin grafting. The control and patient samples were collected and fixed in formaldehyde immediately after resection.
To collect sera, fresh bloods samples were obtained from 22 DM patients, 6 CADM patients and 4 polymyositis (PM) patients. The samples were incubated at room temperature for 30 minutes, then centrifuged at 1500g for 15 minutes. The clinical and laboratory data reported in this study were obtained at the time of serum sampling. Control serum samples were also collected from healthy age- and sex-matched volunteers. All serum samples were stored at –80°C prior to use. Institutional review board approval and written informed consent were obtained before patients and healthy volunteers were entered into this study, according to the Declaration of Helsinki. Patients who had received treatments were excluded.

miRNA extraction from tissue and the PCR analysis of miRNA expression

Small RNAs were extracted from skin sections using miRNeasy FFPE kit (Qiagen, Valencia, CA, USA). Then, RNAs (100ng) were reverse-transcribed into first strand cDNAs with RT^2 miRNA First Strand Kit (Qiagen). For PCR Array, the cDNA was mixed with RT^2 Real-Time SYBR GREEN/ROX PCR Master Mix (Qiagen) and the mixture was added into 96-well RT^2 miRNA PCR Array (Qiagen) that included primer pairs for 88 human miRNAs. PCR was performed on Takara Thermal Cycler Dice (TP800®) following the manufacturer's protocol. The threshold cycle (Ct) for each miRNA was extracted using Thermal Cycler Dice Real Time System ver 2.10B. The raw Ct was normalized using the values of SNORD47, small RNA housekeeping gene stably expressed in the arrays.
For quantitative real-time PCR, Mir-X™ miRNA First-Strand Synthesis Kit (Clontech) was used for cDNA synthesis. The primers for miR-223 or U6 (Takara) and templates were mixed with SYBR Advantage qPCR Premix (Clontech). DNA was amplified for 40 cycles of denaturation for 5 seconds at 95 °C and annealing for 30 seconds at 60 °C. The transcript levels of miR-223 were normalized to those of U6.

miRNA extraction from serum and the PCR analysis of miRNA expression

The miRNA isolation from serum samples was performed with miRNeasy RNA isolation kit (Qiagen) following the manufacturer's instructions, with minor modifications [18]. In brief, 100 (L of serum were supplemented with 5 μL of 5 fmol/(L synthetic non-human miRNA (*C. elegans*, miR-54, Takara) as a control to provide an internal reference for normalization of technical variations between samples. After Qiazol solution (1 mL) was added and mixed well by vortexing, samples were incubated at room temperature for 5 minutes. Aqueous and organic phase separation was achieved by the addition of chloroform and centrifugation. The aqueous phase was applied to RNeasy spin column and RNeasy MinElute spin column. The miRNA was eluted from the column with nuclease-free water.
Mir-X miRNA First-Strand Synthesis Kit (Takara) was used to synthesize first-strand cDNA from the serum-derived miRNAs (about 100 ng). Quantitative real-time PCR with Takara Thermal Cycler Dice (TP800®) used primers and templates mixed with the SYBR Premix. The DNA was amplified for 50 cycles of denaturation for 5 seconds at 95 °C and annealing for 20 seconds at 60 °C. The transcript levels of miR-223 were normalized to those of cel-miR-54 in the same samples.

In situ hybridization

In situ hybridization was performed with 5'-locked digoxigenin-labeled nucleic acid (LNA) probes complementary to human mature miR-223 or a scrambled negative control (Exiqon, Vedbaek, Denmark) [19]. In brief, human skin sections were deparaffinized and deproteinized with protease K for 5 minutes. Slides were then washed in 0.2% glycine in PBS and fixed with 4% paraformaldehyde. Hybridization was performed at 48 °C overnight, followed by blocking with 2% fetal bovine serum and 2% bovine serum albumin in PBS and 0.1%Tween 20 (PBST) for 1 hour. The probe-target complex was detected immunologically by incubating the samples with a digoxigenin antibody conjugated to alkaline phosphatase, which acts on the chromogen nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Roche Applied Science, Mannheim, Germany) for 48 hours. Slides were counterstained with nuclear fast red, and examined under a light microscope (OLYMPUS BX50, Tokyo, Japan).

Immunohistochemical staining

Wax-embedded sections (4 μm thick) were dewaxed in xylene and rehydrated in graded alcohols [20]. Antigens were retrieved by incubation with citrate buffer (pH6) for 5 minutes in a microwave oven. After endogenous peroxidase activity was inhibited, sections were blocked with 5% milk for 30 minutes and then reacted with the antibodies against PKCε (1:40, Santa Cruz Biotech, California, USA) overnight at 4 °C. After excess antibodies were washed out with PBS, samples were incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit antibodies (Nichirei, Tokyo, Japan) for 1 hour. The reaction was visualized using the diaminobenzidine substrate system

(Dojin, Kumamoto, Japan). The slides were counterstained with Mayer's hematoxylin, and examined under a light microscope (OLYMPUS BX50, Tokyo, Japan).

Cell culture

Normal human epidermal keratinocytes (NHEKs) from 3 different donors were purchased from Lonza (Walkersville, MD). NHEKs were cultured in KBM-Gold Basal Medium with KGM-Gold SingleQuot Kit (Lonza) at 37 °C in a 5% CO_2 atmosphere.

Transient transfection

siRNAs were purchased from Dharmacon (Lafayette, CO). Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA) was used as a transfection reagent. One day before transfection, NHEKs were cultured in KGM-Gold so that they were 30-50% confluent at the time of transfection. siRNAs were mixed with the transfection reagent and added to each well containing cells. Then, the NHEKs were incubated for 48-72 hours at 37 °C in 5% CO_2.
miRNA inhibitors were purchased from QIAGEN. For reverse transfection, The miRNA inhibitors were mixed with Lipofectamine RNAiMAX and then added when cells were plated, followed by incubation for 48-96 hours at 37 °C in 5% CO_2 [23, 24].

Cell lysis and immunoblotting

NHEKs were washed with PBS twice and lysed in lysis buffer (Denaturing Cell Extraction Buffer; Biosource International, Camarillo, CA). Aliquots of the cell lysates (normalized for protein concentrations) were subjected to electrophoresis on 10% sodium dodecyl sulfate–polyacrylamide gels and transferred onto PVDF filters. The PVDF filters were blocked in blocking One P buffer (Nacalai Tesque, Kyoto, Japan) for 1 hour and then incubated with primary antibody against PKCε (Sigma-Aldrich Japan, Tokyo, Japan) or β actin (Santa Cruz Biotechnology) overnight. The membranes were washed with Tris-buffered saline and 0.1%Tween 20 (TBS-T), probed with HRP-conjugated secondary antibody for 1 hour, and then washed with TBS-T again. The detection was performed using the ECL system (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's recommendations.

Cell counting

For proliferation assays, cells were plated in 24-well plates and detached from the wells by trypsin treatment. The cell number was counted using Coulter® Particle Counter (Beckman Coulter, Fullerton, CA) [25].

Statistical analysis

The data are expressed as the means ± standard deviation (SD) of at least 3 independent experiments. The statistical analyses were performed using the Mann-Whitney U-test for comparisons of medians. Values of $p<0.05$ were considered to be significant.

Results

The miRNA expression profile in Gottron's papules of DM and CADM

As an initial experiment to determine which miRNAs were involved in the pathogenesis of the cutaneous lesions in patients with DM or CADM, we performed miRNA PCR array analysis, which included 88 miRNAs involved in human cell differentiation and development *(table 1)*. The miRNA expression profiles in the Gottron's papules in the DM and CADM patients were compared with those of normal skin. There were several miRNAs that were overexpressed or suppressed specifically in DM and CADM skin *(table 1)*. Among them, we focused on miR-223, one of the miRNAs which was detected in normal skin but not in DM skin, and which was down-regulated in CADM skin. To confirm the result obtained by the miRNA PCR array, we performed quantitative real-time PCR analysis using specific primers for miR-223. As expected, miR-223 was found to be significantly decreased in DM and CADM compared with normal skin ($p = 0.006$ and 0.003, respectively, *figure 1*), but not in psoriasis skin, which is also characterized by the proliferation of epidermal cells. These results suggest that down-regulation of miR-223 is specific to DM and CADM. In addition, *in situ* hybridization showed that miR-223 expression was not found in the hyperproliferated epidermis of the Gottron's papules in DM *(figure 2figures 2A-B)* and CADM patients *(figure 2C)*, while the signal for miR-223 was evident in the basal layers of normal epidermis *(figure 2D)*.

Table 1. The expression profiles of miRNAs in the skin of DM and CADM as measured with the PCR array

miRNAs down-regulated in DM/CADM skin			
	Normal skin	**DM**	**CADM**
let-7a	-0.44	ND	ND
let-7e	2.81	ND	19.07
let-7g	1.52	ND	ND
miR-21	-2.09	ND	20.41
miR-22	2.27	ND	ND
miR-26a	-2.26	ND	ND
miR-92a	-0.88	ND	35.26
miR-146a	2.58	ND	23.59
miR-146b-5p	2.88	ND	ND
miR-223	1.88	ND	17.11
miR-378	2.19	ND	ND
miRNAs up-regulated in DM/CADM skin			
miR-218	ND	4.59	2.35
miR-503	ND	8.03	10.86

A mixture of equal amounts of miRNAs from 3 normal skin, 3 DM or 3 CADM samples was prepared, and the miRNA expression profile of each disease in vivo was evaluated using PCR array. The raw threshold cycle (Ct) was normalized using the values of small RNA housekeeping gene SNORD47. The ΔΔCt (the raw Ct of each miRNA – Ct of small RNA housekeeping gene) is shown. ND; not detected.

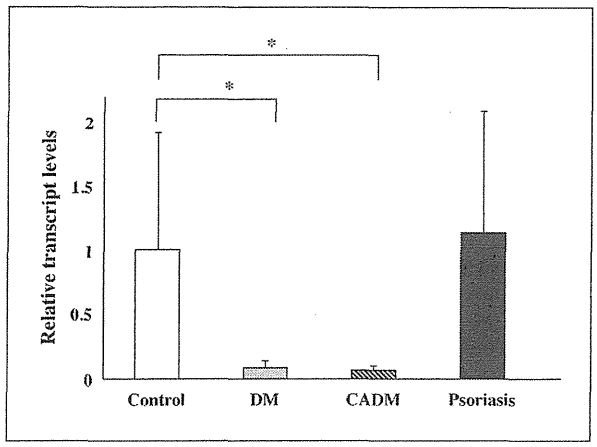

Figure 1. The expression levels of miR-223 in the skin of DM and CADM. The mean relative transcript levels of miR-223 in the tissues from 6 normal skin (NS), 6 DM, 5 CADM and 6 psoriasis patients were determined by quantitative real-time PCR. The bars show the means. The error bars represent SD of +1. The mean value in the NS samples was set at 1. *p<0.05 compared with the values in samples from NS.

Low miR-223 expression leads to cell proliferation via the induction of PKCε in DM and CADM skin

We expected that miR-223 might play a role in the pathogenesis of the cutaneous lesions of DM and CADM. According to TargetScan, the miRNA target gene prediction database (version 5.1, http://www.targetscan.org/), we found PKCε was one of the putative target genes of miR-223. Because PKCε was previously implicated as an effective promoter of keratinocyte growth *in vivo* and *in vitro* [26, 27], we hypothesized that decreased miR-223 expression would cause the induction of PKCε, resulting in the increased keratinocyte proliferation seen in the epidermis of Gottron's papules.

The immunohistochemical analyses revealed that the protein expression of PKCε in the hyperproliferated epidermis of Gottron's papules in DM patients was increased compared to that in normal skin *in vivo (figure 3)*. Next, to confirm that PKCε was a target of miR-223, NHEKs were transfected with miR-223 inhibitor, and the expression of PKCε was evaluated by immunoblotting. The inhibition of miR-223 *in vitro* resulted in a significant increase in the protein expression of PKCε ($p = 0.049$, *figure 4A*). Our results indicated that decreased miR-223 results in the overexpression of the PKCε protein in DM and CADM skin.

We next investigated whether miR-223 is involved in the keratinocyte proliferation via PKCε. A specific inhibitor of miR-223 significantly induced cell proliferation significantly ($p<0.001$, *figure 4B*). On the other hand, when PKCε was knocked down by a specific siRNA ($p = 0.016$, *figure 4C*), we observed a significant decrease in the cell number ($p = 0.021$, *figure 4D*). Taken together, these findings suggest that the abnormal keratinocyte proliferation in the epidermis of Gottron's papules may be caused by decreased miR-223 expression and subsequently increased levels of PKCε.

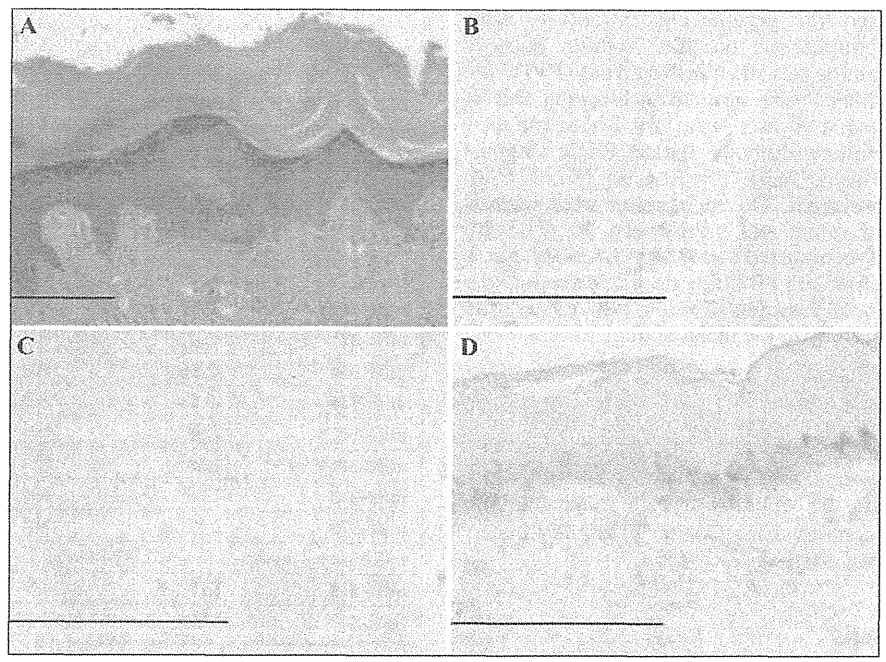

Figure 2. *In vivo* expression of miR-223 in the skin of DM and CADM. A) Representative histopathological findings of Gottron's papule. Skin biopsy sample was obtained from Gottron's papule of a DM patient and was stained with hematoxylin and eosin. Scale bar = 100 μm. **B-D)** *In situ* detection of miR-223 in paraffin-embedded, formalin-fixed tissues of DM skin **(B)**, CADM skin **(C)** and normal skin **(D)**. The Nuclei were counterstained with nuclear fast red. miR-223 is stained dark brown. Representative results from three independent experiments are shown. Scale bar = 100 μm.

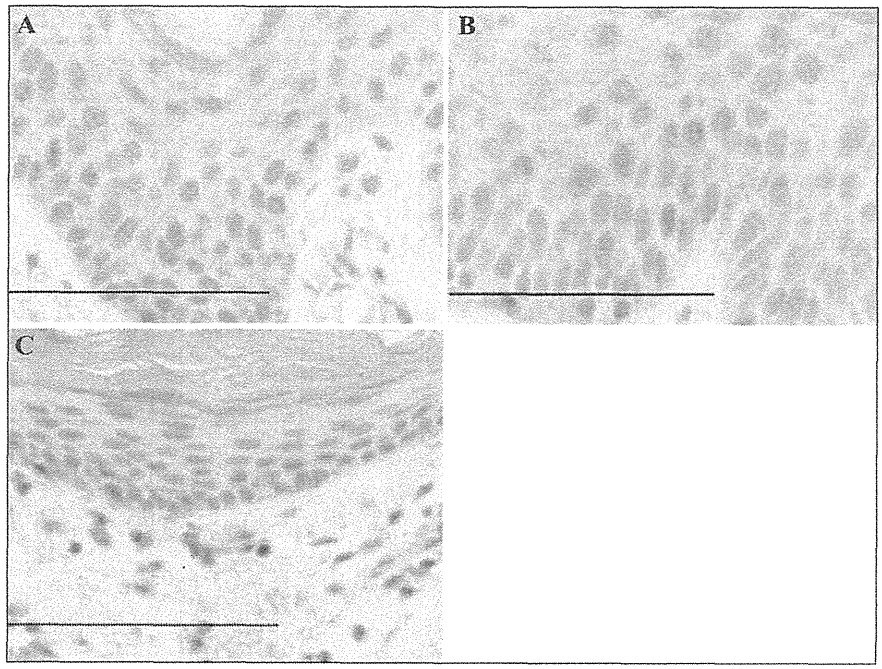

Figure 3. The expression pattern of PKCε in DM skin. Immunostaining for PKCε was performed using the tissue sections of DM skin (**A**), CADM skin (**B**), and normal skin (**C**). Scale bar = 100 μm.

Serum levels of miR-223 in DM patients

We also determined the serum concentration of miR-223 by quantitative real-time PCR and evaluated the possibility that the serum miR-223 levels can be a disease marker for DM. Serum samples were obtained from 22 patients with DM, 6 patients with CADM, 4 patients with PM and 19 healthy control subjects. The serum miR-223 levels were significantly decreased in patients with PM/DM/CADM compared with healthy controls by Mann-Whitney U-test ($p = 0.015$, *figure 5*). When patients were divided into groups based on the disease subtype, the serum miR-223 levels of CADM patients were significantly decreased compared with healthy controls ($p = 0.015$). On the other hand, although the miR-223 levels also tended to be decreased in patients with DM and those with PM, there were no statistically significant differences between controls and DM patients or between controls and PM patients *(figure 5)*. We next examined the correlation between the serum miR-223 levels and the clinical features of patients with PM/DM/CADM. As shown in *Table 2*, the disease duration (between symptom onset and the first visit to the hospital) was significantly shorter in patients with decreased miR-223 levels than in those with normal levels (2.8 *vs* 8.6 months, $p = 0.032$), indicating that those with decreased miR-223 levels may have more severe symptoms. Additionally, although there was no statistically significant difference ($p = 0.29$), the frequency of Gottron's papules tended to be increased in patients with reduced serum levels of miR-223 compared to those with normal levels, which is consistent with the array results indicating that miR-223 expression is reduced in Gottron's papules *in vivo*.

Discussion

This is the first study that has examined the miRNA expression specifically changed in DM skin. We herein demonstrated a role for the miR-223-PKCε pathway in keratinocyte proliferation and its contribution to the pathogenesis of Gottron's papules by uncovering three major findings.

We identified several miRNAs that were specifically overexpressed or suppressed in DM and CADM skin compared with normal skin by PCR array. Abnormal expression of miR-223 has been observed in a number of human diseases, such as rheumatoid arthritis, viral infections, sepsis, multiorgan failure, and cancer [28]. This is the first study that has indicated a role for miR-223 in DM. As a limitation of this study, because the array analysis was performed as a single experiment, the statistical significance could not be evaluated. Therefore, we confirmed the array result using real-time PCR and *in situ* hybridization, which also indicated the down-regulation of miR-223 expression in DM/CADM.

Second, in this study, we also found a new miRNA-mRNA target interaction in DM skin: the down-regulation of miR-223 leads to the overexpression of PKCε in keratinocytes. In addition, our results indicated that PKCε regulates keratinocyte growth, consistent with a previous report [26, 27]. PKCε is a molecule comprising 737 amino acids, and is found in diverse tissues, including neuronal, heart, immune, retinal, endothelial and epidermal cells. Our results revealed that PKCε is mostly localized in the proliferative basal layers in the epidermis, and suggested that the acanthosis and hyperkeratosis in Gottron's papules may be

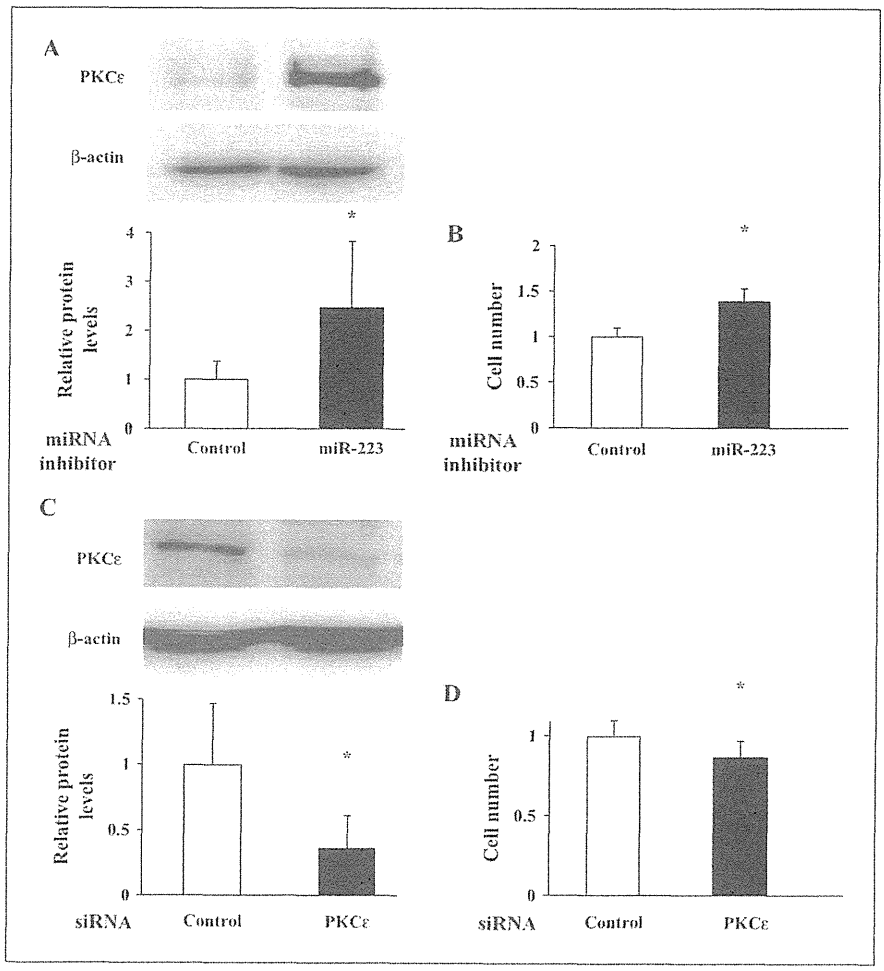

Figure 4. The miR-223-PKCε pathway regulates the proliferation of keratinocytes. A) (upper panel) NHEKs were incubated at a density of 1.0×10^5 cells/well in 6-well culture plates, and were transfected with control or miR-223 inhibitor for 72 hours. Cell lysates were subjected to immunoblotting with antibodies against PKCε and β-actin. The representative results from 3 independent experiments using different donors are shown. (lower panel) The PKCε levels quantitated by scanning densitometry and corrected for the β-actin levels in the same samples are shown. *$p<0.05$ compared with the value in cells transfected with the control inhibitor (1.0) (n = 3). **B)** NHEKs were incubated at a density of 1.0×10^4 cells/well in 24-well culture plates and transfected with control or miR-223 inhibitor for 72 hours. The number of cells was counted with Coulter® Particle Counter. The means and SD from 3 independent experiments are shown. * $p<0.05$ compared with the values in the cells transfected with the control inhibitor. **C,D)** NHEKs from three different donors were transfected with control siRNA or siRNA specific for PKCε After 48 hours, **(C)** cell lysates were subjected to immunoblotting with antibodies against PKCε and β-actin. The representative results from 3 independent experiments using different donors are shown. The band intensities were analyzed as described in *figure 4A*. **(D)** The cells were counted as described in *figure 4B*.

caused by miR-223 down-regulation via the induction of PKCε.

Finally, we performed the first investigation of the serum miRNA levels in DM patients. There have been a few reports showing that miR-223 is detectable and quantifiable in serum. For example, Wang *et al.* reported that the serum miR-223 level was significantly reduced in septic patients [30]. Our results indicate that the miR-223 concentration was significantly decreased in patients with PM/DM/CADM, especially in CADM patients. CADM is characterized by skin lesions, and is not accompanied by the muscle weakness seen in DM. Thus, these results indicate that the serum miR-223 level is decreased in association with cutaneous involvement of the disease. Furthermore, patients with decreased serum miR-223 levels tended to visit hospital earlier than those with normal miR-223 levels. These results indicate that patients with decreased miR-223 levels have more severe symptoms. Thus, the serum miR-223 level might serve as new biomarker for CADM: The diagnosis of CADM is sometimes difficult, especially in the absence of myositis or lung involvement. The miR-223 down-regulation may be useful for the differential

Table 2. The association of the serum miR-223 levels with the clinical features in DM patients

Clinical and serological features	Patients with normal miR-223 levels(n = 15)	Patients with decreased miR-223 levels(n = 18)
Age at onset (mean years)	53.9	55.2
Duration of disease (mean months)	8.6*	2.8
SKIN ERUPTIONS		
Gottron's papules	64.3	80.0
Heliotrope rash	54.5	61.5
ORGAN INVOLVEMENT		
Muscle weakness	66.7	78.6
Arthralgia	0.0	22.2
Dysphagia	11.1	25.0
Lung involvement	23.1	30.7
Cancer	23.1	7.6
LABORATORY FEATURES		
Elevated CK	64.3	62.5
Elevated myoglobin	76.9	100.0
Elevated aldolase	45.5	50.0
Elevated IgG	25.0	26.6
ANA SPECIFICITY		
Anti Jo-1	25.0	0.0
Anti-U1 RNP	0.0	22.2

*Unless indicated, the values are percentages. CK, creatinine kinase; ANA, anti-nuclear antibody; anti Jo-1, anti-Jo-1 antibody; anti-U1 RNP, anti-U1 RNP antibody. *p<0.05, compared with the values in patients with decreased serum miR-223 levels.*

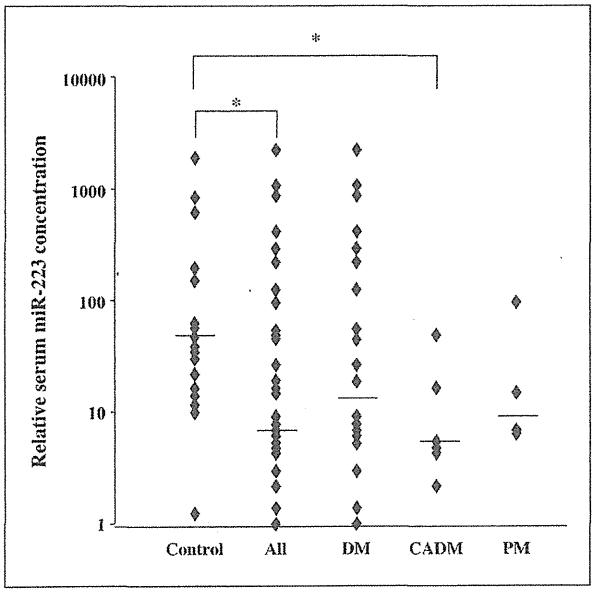

Figure 5. Serum concentrations of miR-223 in patients with PM, DM or CADM. The serum miR-223 levels were measured by quantitative real-time PCR as described in 'Materials and Methods'. The miR-223 concentrations are shown on the ordinate. The minimum value in DM patients was set at 1. The bars show medians. *p values were determined using Mann-Whitney U-test. all; patients with PM, DM and CADM.

diagnosis of other skin diseases. Comparison of miR-223 levels between CADM and other diseases in an increased number of patients is needed in the future.

Taken together, our results suggest that miR-223 expression is down-regulated in Gottron's papules of patients with DM/CADM. The down-regulated miR-223 induces PKCε expression, which results in the keratinocyte proliferation in Gottron's papules. Our study of the regulatory mechanisms of keratinocyte proliferation by miRNA may shed light on the pathogenesis of this disease. Although steroid ointment is currently used for the treatment of the cutaneous lesions of DM/CADM, it is sometimes ineffective. Further studies may lead to the development of new treatments using miRNA. ■

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Uncited references

[12-16, 21, 22, 29].Q1

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