Original Investigation

Clinical and Histologic Analysis of the Efficacy of Topical Rapamycin Therapy Against Hypomelanotic Macules in Tuberous Sclerosis Complex

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IMPORTANCE Tuberous sclerosis complex (TSC) is an autosomal dominant disorder leading to the aberrant activation of the mammalian target of rapamycin complex 1. Although the efficacy of mammalian target of rapamycin complex 1 inhibitors against tumors in patients with TSC, including facial angiofibroma, has been well investigated, their efficacy against hypomelanotic macules in patients with TSC is unknown.

OBJECTIVES To evaluate objectively the efficacy of topical rapamycin treatment of hypomelanotic macules in patients with TSC and to elucidate the mechanisms of how rapamycin improves the macules.

DESIGN, SETTING, AND PARTICIPANTS We performed a prospective, baseline-controlled trial of 6 patients with TSC and hypomelanotic macules in non–sun-exposed and sun-exposed skin at the Department of Dermatology, Osaka University, from August 4, 2011, through September 27, 2012. Rapamycin gel, 0.2%, was applied to the lesions twice a day for 12 weeks. Histologic examinations and blood tests were conducted at the start and completion of treatment. Blood rapamycin levels were analyzed at completion.

EXPOSURES Topical rapamycin treatment for hypomelanotic macules.

MAIN OUTCOMES AND MEASURES Objective evaluation of rapamycin treatment of hypomelanotic macules in TSC with δ-L (L indicates the brightness of the color) levels on spectrophotometry at the start and completion (12 weeks) of treatment and at 4 and 12 weeks after discontinuation of treatment (16 and 24 weeks, respectively).

RESULTS Improvement of hypomelanotic macules (in δ-L values) was significant at 12 (mean [SD], 2.501 [1.694]; $P < .05$), 16 (mean [SD], 1.956 [1.567]; $P < .01$), and 24 (mean [SD], 1.836 [1.638]; $P < .001$) weeks. Although efficacy tended to be prominent in sun-exposed skin, we did not observe significant differences (in δ-L values) between sun-exposed and non–sun-exposed skin at 12 (mean [SD], 1.859 [0.629] and 3.142 [2.221], respectively), 16 (mean [SD], 1.372 [0.660] and 2.539 [2.037], respectively), and 24 (mean [SD], 1.201 [0.821] and 2.471 [2.064], respectively) weeks. No adverse events were observed, and rapamycin was not detected in the blood of any patient. Electron microscopic analysis of hypomelanotic macules revealed that topical rapamycin treatment significantly improved the uniformity of the melanosome numbers in the TSC melanocytes (pretreatment macules: mean [SD], 25.71 [21.90] [range, 5-63]; posttreatment macules: mean [SD], 42.43 [3.60] [range, 38-49]; $P < .001$). Moreover, rapamycin treatment induced the recovery of melanosomes in TSC-knocked-down melanocytes from depleted amounts (mean [SD], 16.43 [11.84]) to normal levels (mean [SD], 42.83 [14.39]; $P < .001$).

CONCLUSIONS AND RELEVANCE Topical rapamycin treatment was effective and safe against hypomelanotic macules arising from TSC. This efficacy of rapamycin was corroborated as stemming from the improvement of impaired melanogenesis in TSC melanocytes.

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Editorial

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that causes multiple hamartomas, epilepsy, autism, and hypopigmented macules. Tuberous sclerosis complex is caused by mutations in the *TSC1* gene (OMIM 605284)[1] or the *TSC2* gene (OMIM 191092)[2]; the genes encode hamartin and tuberin, respectively. The hamartin-tuberin complex downregulates the mammalian target of rapamycin complex 1 (mTORC1).[3,4] The constitutive activation of mTORC1 that results from the abnormality of the *TSC1* or *TSC2* gene is associated with abnormal cellular proliferation, which causes TSC-related hamartomas. Recent reports suggest that mTORC1 inhibitors, such as rapamycin, may be effective for the treatment of TSC-related tumorigenesis, including facial angiofibromas.[5] The efficacy of topical rapamycin treatment for facial angiofibroma in patients with TSC also has been reported.[6-14] With regard to hypomelanotic macules, which are resistant to effective treatment, the mechanism by which mTORC1 affects melanogenesis is also still unknown, although hypomelanotic macules are one of the major diagnostic criteria for TSC.[15,16] Hypomelanotic macules in TSC are very important because they arise at birth or in early infancy, resulting in the early diagnosis of TSC. In addition, hypomelanotic macules appearing on the face are cosmetically displeasing and require treatment. Ohguchi et al[17] and Hah et al[18] reported that rapamycin upregulated microphthalmia-associated transcription factor, a master regulator of melanogenesis, in B16 and MNT-1 melanoma cells. Jóźwiak and Galus[19] reported the inhibition of microphthalmia-associated transcription factor by mTOR in TSC. Ho et al[20] reported that TORC1 is related to melanogenesis through the activation of the melanogenic enzyme and the formation of mature melanosomes. In addition, Murase et al[21] reported that autophagy, which is regulated by mTORC1, is involved in the degradation of melanosomes in keratinocytes. Recently, Wataya-Kaneda et al[22] reported 2 cases of hypomelanotic macules in patients with TSC who recovered after the topical administration of rapamycin. The mTORC1 inhibitors, such as rapamycin, might be effective not only for tumors but also for hypomelanotic macules in patients with TSC.

The aims of this study were to evaluate objectively and precisely the efficacy of topical rapamycin treatment and to investigate the histologic and cytologic effects of topical rapamycin on TSC-related hypomelanotic macules. To assess the findings objectively, we evaluated the efficacy of rapamycin with the use of a spectrophotometer (CM-700d; Konica Minolta). In addition, the specimens of hypomelanotic macules before and after rapamycin treatment were examined by electron microscopy. To corroborate the effect of rapamycin on the melanogenesis that was observed in the rapamycin-treated hypomelanotic macules in TSC, the effect of rapamycin on *TSC2*-knocked-down melanocytes (TSC-model melanocytes) was examined. We also compared the efficacy of topical rapamycin treatment on hypomelanotic macules in sun-exposed and non-sun-exposed skin in patients with TSC to investigate the effect of sun exposure.

Methods

Ethical Consideration

This study was approved by the ethics committee of the Osaka University Faculty of Medicine (approval 10339) and was disclosed to the University Hospital Medical Information Network (report number UMIN000006108). All the patients volunteered for this trial and signed written informed consent agreements. On behalf of the children, written informed consent was obtained from each legal representative.

Study Design

This study was a prospective, baseline-controlled trial. Six patients with definitive TSC and hypomelanotic macules in non-sun-exposed and sun-exposed skin who wanted to participate in this trial were enrolled from among 250 outpatients at the Department of Dermatology, Osaka University. Rapamycin gel, 0.2%, was applied to hypomelanotic macules on non-sun-exposed and sun-exposed (on the face in 5 patients and above the knee in 1 patient) skin on each patient twice a day for 12 weeks. Assessment of each patient was performed once a month during the 12 weeks of rapamycin treatment and at 4 and 12 weeks after discontinuation of the treatment (16 and 24 weeks, respectively). Blood rapamycin levels were analyzed at the end of treatment using liquid chromatography-electrospray ionization mass spectrometry (detection limit, 0.6 ng/mL).

Powder prepared from 2-mg rapamycin tablets (sirolimus [Rapamune]) was mixed with a 100-mg gel to a concentration of 0.2% rapamycin. We enrolled 6 patients aged 3 to 33 (mean age, 11.7) years who were diagnosed as having definitive TSC according to the diagnostic criteria update.[16] Four of the 6 patients were male; 2 were female. All non-sun-exposed skin areas were on the trunk except for 1 thigh, and all the sun-exposed skin areas were on the face except for 1 knee (**Table**).

To determine the treatment efficacy objectively, assessment was performed using a spectrophotometer to measure the δ-L value of each hypomelanotic macule before and after treatment.[23-26] Decreased δ-L values reflected improved hypomelanotic macules.

Evaluation of Rapamycin Efficacy

Immunohistochemical Examinations

Skin specimens of hypomelanotic macules, obtained before treatment from 6 participants and after treatment from 1 participant, were fixed in buffered 10% formalin and embedded in paraffin. The paraffin-embedded tissue slides were stained with hematoxylin-eosin and Fontana-Masson. After the antigen was retrieved by boiling each specimen in an oil bath for 15 minutes in a 10mM TRIS/1mM EDTA buffer (pH, 9.0), the slides were also incubated with a goat anti-melan A monoclonal antibody (1:50) (Dako) at 4°C overnight and with a biotinylated-link universal antibody, conjugated streptavidin-alkaline phosphatase, and a fuchsin substrate-chromogen buffer. These slides were observed under a microscope.

Table. Characteristics of the Patients and the Treated Lesions

Patient No./Sex	Target Sites to Be Treated		Histologic Examination Result		No. of Melanosomes in Each Melanocyte	
	Sun-Exposed Skin	Non-Sun-Exposed Skin	Melan A Staining	Fontana-Masson Staining	Mean	*P* Value for Homogeneity[a]
1/M	Face	Trunk	Positive	Positive	28.60	.01
2/M	Face	Trunk	Positive	Positive	18.67[b]	.17
3/F	Face	Thigh	Positive	Positive	19.20	.03
4/M[c]	Face	Trunk	Positive	Positive		
5/M	Face	Trunk	Positive	Positive	31.10	.06
6/F	Knee	Trunk	Positive	Positive	25.71	.049

[a] $P < .05$ was considered statistically significant.

[b] Indicates count was significantly small.

[c] We were unable to obtain a specimen for electron microscopic examination from patient 4.

Electron Microscopic Examination of the Skin Tissue

Punched skin samples (1 mm each) from the hypomelanotic macules in patients with TSC before and after treatment were fixed in 2.5% glutaraldehyde in a 0.1M phosphate buffer (pH, 7.4) at 4°C. Postfixation took place in 1% osmium tetroxide in a 0.1M phosphate buffer (pH, 7.4) for 1 hour, after which the samples were dehydrated in an ethanol dilution series and embedded in epoxy resin (Quetol-812; Nisshin EM). Semithin sections (0.5 µm) were stained with toluidine blue O, and ultrathin sections (0.1 µm) were stained with saturated uranyl acetate and lead citrate. Sections on copper mesh were examined using a commercially available electron microscope (JEM-1200EX; Jeol).

Melanin Counts in Melanocytes

Seven melanocytes were randomly selected in each sample for counting. The number of melanosomes in each melanocyte was counted directly at a magnification ×8000 and then summarized in a graph.

RNA Interference

For the short interfering RNA (siRNA) experiments, double-stranded RNA duplexes composed of 21-nucleotide sense and antisense oligonucleotides (Cosmo Bio Ltd) were synthesized. The RNA oligonucleotides used for targeting human *TSC2* in this study were 5′-CGAACGAGGUGGUGUCCUATT-3′ for *TSC2* sense and 5′-UAGGACACCACCUCGUUCGTT-3′ for *TSC-2* antisense.

Transfection of Short Interfering *SC2* and Rapamycin Stimulation With siRNA-Transfected Melanocytes

Human neonatal epidermal melanocytes from a moderately pigmented donor (HEMn-MP) were purchased from Invitrogen and cultured in medium 254 (M-254-500; Gibco) with the addition of human melanocyte growth supplement (Invitrogen) at 37°C in an atmosphere of 5% carbon dioxide. The melanocytes were used in passages 6 through 8. We seeded HEMn-MP cells onto 6-well plates at a density of 5×10^5 cells/well 12 hours before transfection. Transfection reagent (Lipofectamine 2000; Invitrogen) was used with 30nM siRNA according to the manufacturer's instructions. The efficacy of the transfection was verified with reverse transcription-polymerase chain reaction at 48 hours after siRNA transfection; HEMn-MP cells were stimulated with 30nM rapamycin (Calbiochem) for 72 hours.

Electron Microscopic Examination of Cultured Cells

After being washed 3 times with phosphate-buffered solution, cultured cells were fixed with 0.5% glutaraldehyde in a 0.1M phosphate buffer (pH, 7.4) at 4°C for 15 minutes at room temperature. After being washed 3 times with phosphate-buffered solution, cells were fixed with 1% osmium tetroxide in phosphate-buffered solution for 3 hours at 4°C. After they were rinsed with water to remove the osmium tetroxide, the cells were dehydrated in a sequential ethanol dilution series. After infiltration in a 1:1 mixture of epoxy resin and ethanol, the cells were embedded in epoxy resin, drained for 60 minutes by inverting the culture dishes on a paper towel, and polymerized overnight at 60°C in a vacuum oven. Ultrathin sections prepared in the same manner as described for the tissue sample were examined with an electron microscope, and the number of melanosomes in each randomly selected melanocyte was counted directly.

Statistical Analysis

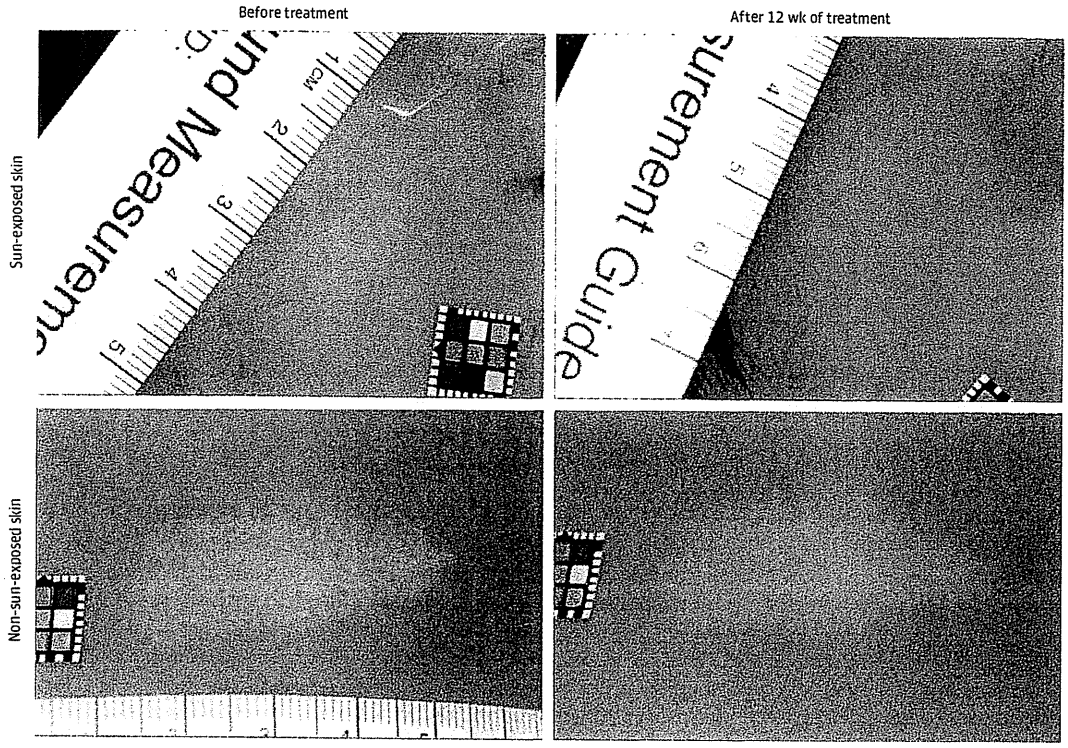
We used a nonparametric approach because the data were not normally distributed. To compare the homogeneity and the mean number of melanosomes before and after rapamycin treatments, we used a Levene test[27] and Wilcoxon rank sum test, respectively. To compare the improvements in the hypomelanotic macules before and after the topical rapamycin treatment, we used simulation-based multiple comparisons based on generalized estimating equations. $P < .05$ was considered significant.

Results

Clinical Improvement of Hypomelanotic Macules

The patient information, treated lesions, and histologic characteristics are summarized in the Table. We have included representative photographs of treatment in sun-exposed and non-sun-exposed skin before and after treatment. The photographs demonstrate that topical rapamycin treatment improved the

Figure 1. Clinical Features of Hypomelanotic Macules in Patient 1



Hypomelanotic macules in sun-exposed and non-sun-exposed skin before and after 12 weeks of topical rapamycin treatment. Color squares are used to correct the difference in color balance of each machine and to keep coherence of real color.

hypomelanotic macules and that the sun-exposed skin improved more than the non-sun-exposed areas (**Figure 1**). In this trial, blood tests were performed on all participants before and after treatment to examine the systemic influence. Blood rapamycin concentration was also analyzed. No adverse effects were observed in any participant, and the rapamycin concentration in the blood was lower than the detection limit (0.6 ng/mL).

Improvement of δ-L Spectrophotometry Values

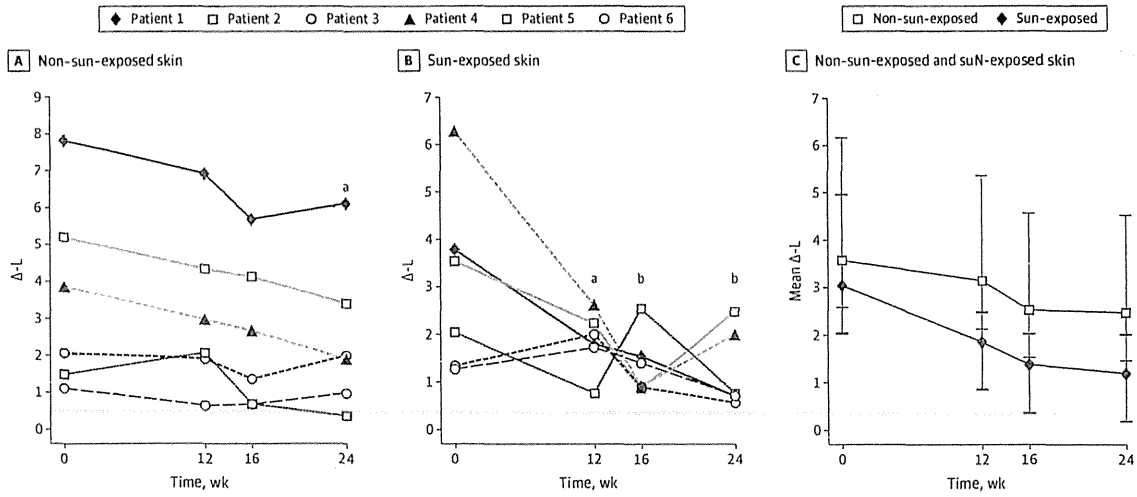
Five of 6 patients demonstrated lower δ-L values at the end of treatment than at initiation, with no significant difference in non-sun-exposed skin at the end of treatment (mean [SD] at 12 weeks, 3.142 [2.221]). However, at 4 weeks after discontinuation of treatment (16 weeks), δ-L values for the non-sun-exposed skin decreased significantly (mean [SD], 2.539 [2.037]; $P = .046$). The significant decline in δ-L values continued for 12 weeks after discontinuation of treatment (mean [SD] at 24 weeks, 2.471 [2.064]; $P < .01$) (**Figure 2A**). Unlike the non-sun-exposed skin, the δ-L values for the sun-exposed skin indicated significant differences at the completion of treatment (mean [SD], 1.859 [0.629]; $P = .046$) and 4 and 12 weeks after discontinuation of treatment (mean [SD], 1.372 [0.660] and 1.201 [0.821], respectively; $P < .01$ for both) (Figure 2B).

To assess the effect of sun exposure on rapamycin treatment, we compared the mean δ-L values for the 6 patients between the non-sun-exposed and sun-exposed skin. Although the mean δ-L values in the sun-exposed skin were lower than those for the non-sun-exposed skin at all points, including at treatment initiation (mean [SD] at initiation, 3.049 [1.912] vs 3.589 [2.580]; at 12 weeks, 1.859 [0.629] vs 3.142 [2.221]; at 16 weeks, 1.372 [0.661] vs 2.539 [2.037]; and at 24 weeks, 1.201 [0.822] vs 2.471 [2.064]), and although both graphs were approximately parallel, we found no significant difference between the δ-L values for the non-sun-exposed and sun-exposed skin (Figure 2C). This result indicated that sun exposure did not affect the efficacy of the topical rapamycin therapy.

Histochemical Examinations of Hypomelanotic Macules

To corroborate the efficacy of rapamycin treatment, we undertook histologic investigations of the TSC-related hypomelanotic macules before and after treatment. Specimens of hypomelanotic macules before treatment were obtained from all 6 participants voluntarily, but an after-treatment specimen was obtained from only patient 6 because the remaining 5 patients refused biopsy of their healed lesions. Six pretreatment skin specimens, 1 posttreatment specimen, and 1 con-

Figure 2. Improvement of Skin Color Measured by Spectrophotometer



A, Change in δ-L values for each patient in non-sun-exposed skin. Decline was significant at 16 and 24 weeks. B, Change in δ-L values for each patient in the sun-exposed skin. Change was significant at 12, 16, and 24 weeks. C, Changes in the mean δ-L values in the non-sun-exposed and sun-exposed skin. Differences between areas were not significant. Whiskers indicate SD. $P < .05$ was considered significant. Zero weeks indicates initiation of rapamycin treatment; 12 weeks, discontinuation of treatment; and 16 and 24 weeks, posttreatment observations.

[a] $P < .05$.

[b] $P < .01$.

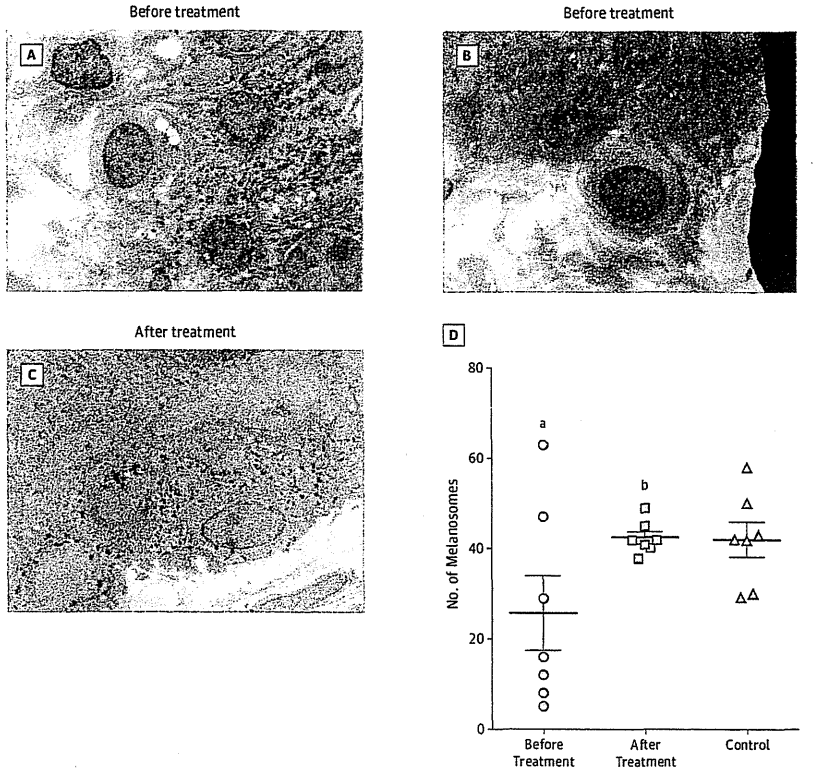
trol specimen were stained with Fontana-Masson and antimelan A antibody. The pretreatment and posttreatment specimens of hypomelanotic macules in patients with TSC demonstrated melan A-positive cells, as did the control skin specimen (eFigure 1A, C, and E in the Supplement). However, in the pretreatment TSC specimens, we observed dispersed faint Fontana-Masson staining. By contrast, as with the control samples, higher and darker Fontana-Masson-stained granules were detected in the posttreatment TSC specimens than in pretreatment specimens (eFigure 1B, D, and F in the Supplement). These results indicated that melanocytes were present but melanin granule levels were decreased in the TSC-related hypomelanotic macules, and topical rapamycin therapy increased the volume of melanin granules observed by microscope in the basal layers.

Electron Microscopic Examinations of Hypomelanotic Macules

Electron microscopic examinations suggested that in TSC hypomelanotic macules, abnormal melanogenesis might occur. To examine the precise abnormality of the melanogenesis, we assessed the numbers and morphologic features of the melanocytic melanosomes in the TSC hypomelanotic macules using an electron microscope. We examined 5 pretreatment samples and 1 posttreatment sample. Before rapamycin treatment, all the melanosomes observed in the samples were well matured at stage III or stage IV. The number of stages III and IV melanosomes varied between melanocytes. Even within the same hypomelanotic lesion from the same patient, some areas had extreme contrasts of few and many melanosomes (**Figure** 3A and B). By contrast, in posttreatment TSC hypomelanotic macules, the numbers of melanosomes in the melanocytes became uniform and were equal to those in the control melanocytes (Figure 3C).

To confirm these findings, 7 melanocytes were selected at random from each sample, and we counted the numbers of pretreatment and posttreatment melanosomes in each melanocyte from patient 6 in microscopic images at a magnification of ×8000. The numbers of melanosomes in the melanocytes in the pretreatment TSC hypomelanotic macules ranged from 5 to 63. By contrast, the numbers of melanosomes in the posttreatment melanocytes ranged from 38 to 49. The melanosomes in the normal control skin sample ranged from 29 to 58. The range of melanosomes in the melanocytes from the pretreatment TSC hypomelanotic macules was significantly broader than the range from the control specimen ($P = .02$). By contrast, the range of melanosomes in the melanocytes from the posttreatment TSC hypomelanotic macules was narrower than that for the pretreatment macules ($P = .049$) (Figure 3D). The numbers of melanosomes in each melanocyte from the pretreatment hypomelanotic macules obtained from the remaining 4 participants were also examined. Three of the 5 patients' pretreatment specimens exhibited significant variation in the numbers of melanosomes. One specimen (patient 2) that did not show a difference in variability reflected the significantly small number of melanosomes in each melanocyte (range, 1-65; $P = .17$) (Table and eFigure 2 in the Supplement). Given that the keratinocytes had abnormalities in the number and morphologic features of melanosomes similar to those of their adjacent melanocytes (Figure 3A), we believe that melanin had been transferred to the adjacent keratinocytes.

Figure 3. Electron Microscopic Examinations of Melanosome Numbers in Hypomelanotic Macules From Patients With Tuberous Sclerosis Complex Before and After Rapamycin Treatment



A, Melanocytes with many melanosomes before treatment. B, Melanocytes with few melanosomes before treatment. C, Melanocytes after treatment (derived from 1 patient). Original magnification ×4000. D, Statistical analysis of homogeneity of melanosome numbers in the melanocytes before and after rapamycin treatment and in the control skin specimen. Data markers indicate melanosome numbers in each melanocyte; bold horizontal lines, mean value of the numbers; and limit lines, SD.

[a] $P = .049$, compared with control.

[b] $P = .02$, compared with pretreatment.

These results indicate that in hypomelanotic macules in patients with TSC, melanocytes existed, melanin transfer from melanocyte to keratinocyte was normal, and melanogenesis in melanocytes was impaired. As a result, maturation of the melanosomes was completed, but the numbers of melanosomes ranged widely between melanocytes, and topical rapamycin treatment reduced this range.

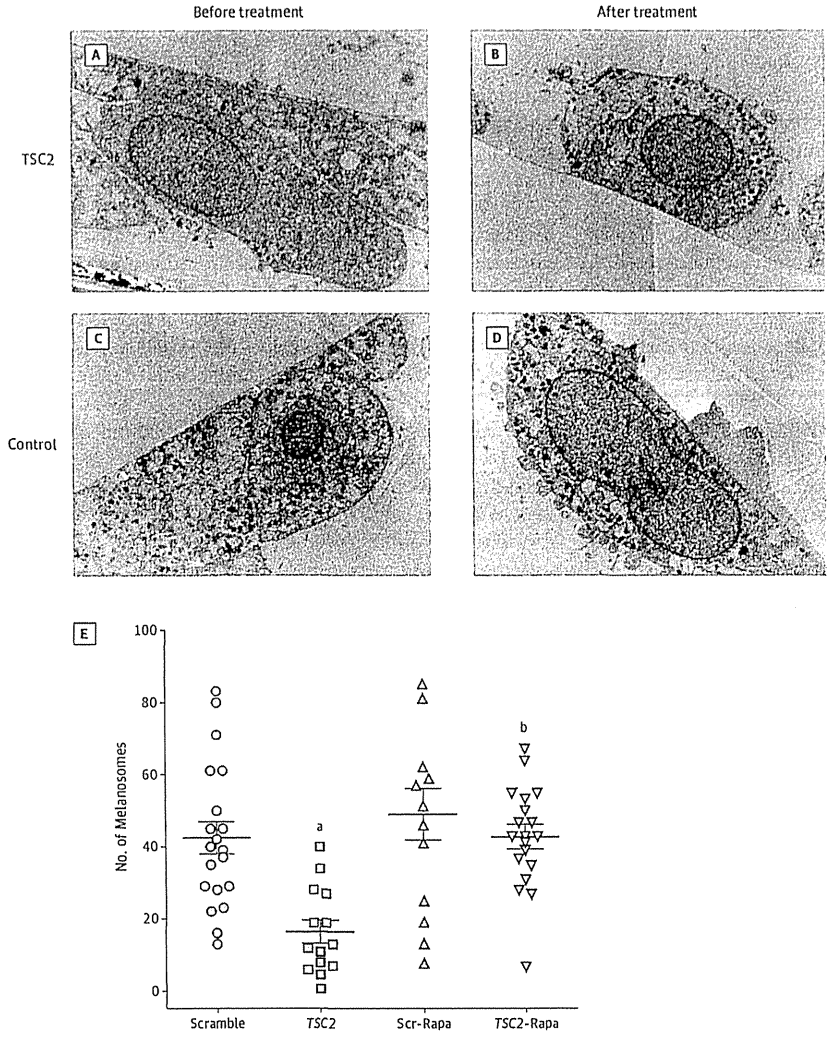
Electron Microscopic Examination of Cultured TSC-Knocked-Down Melanocytes

To corroborate the characteristics of TSC melanocytes and the efficacy of rapamycin, *TSC2*-knocked-down melanocytes (ie, TSC-model melanocytes) were prepared using short interfering *TSC2*, and the effects of rapamycin were examined. Because the effectiveness rate of the *TSC2*-model melanocytes was approximately 70%, we found verification of the variability to be difficult. However, many melanocytes with only a small number of melanosomes were observed in the TSC-model melanocytes. After rapamycin treatment, the number of melanosomes in the TSC-model melanocytes increased (**Figure 4**A and B), although no change occurred in the control melanocytes (Figure 4C and D). To confirm the effect of the rapamycin, the numbers of melanosomes in the TSC-model and control melanocytes were counted. The TSC-model melanocytes had significantly fewer melanosomes (mean [SD], 16.43 [11.84]) than did the control melanocytes (mean [SD], 42.5 [20.1]; $P < .001$). However, after rapamycin treatment, the number of melanosomes in the TSC-model melanocytes significantly increased (mean [SD], 42.83 [14.39]; $P < .001$) and approached the numbers in the control melanocytes (Figure 4E). These results corroborated that abnormal melanogenesis had occurred in the TSC-model melanocytes and that rapamycin reduced the abnormalities without affecting normal cells.

Discussion

Tuberous sclerosis complex is an autosomal dominant disorder caused by the inactivation of 1 of 2 tumor suppressor genes, *TSC1*[1] or *TSC2*,[2] leading to the aberrant activation of mTORC1.[3,4] Thus, mTORC1 inhibitors, such as rapamycin, have been used to treat patients with TSC. The mechanism of tumorigenesis via mTORC1 is well investigated, and the efficacy of rapamycin for treating tumors in TSC has been already reported.[5,28-30] By contrast, only 1 study of hypomelanotic macules has been reported,[22] and the mechanisms and therapeutic approaches to hypomelanotic macules in TSC remain unknown, although these macules are a major feature of TSC.[15,16] In this report, we treated hypomelanotic macules in 6 patients with definitive TSC and objectively verified the efficacy of a topical rapamycin treatment using a spectrophotometer.

Figure 4. Electron Microscopic Examination of Melanosome Numbers in Tuberous Sclerosis Complex (TSC) Model and Control Melanocytes Before and After Rapamycin Treatment



A, TSC-model melanocytes before treatment. B, TSC-model melanocytes after treatment. C, Control melanocytes before treatment. D, Control melanocytes after treatment. Original magnification ×8000. E, Statistical analysis of the mean numbers of melanosomes in each melanocyte. *TSC2* and tsc2 indicate short interfering (si) *TSC2* knocked-down melanocyte (TSC model melanocyte) before rapamycin treatment. *TSC2*-rapa indicates si-*TSC2* knocked-down melanocyte after rapamycin treatment; scramble, control melanocyte before rapamycin treatment; and scr-rapa, control melanocyte after rapamycin treatment. Data markers indicate melanosome numbers in each melanocyte; bold horizontal lines, mean value of the numbers; and limit lines, SD.

[a] $P < .001$ compared with scramble.

[b] $P < .001$ compared with *TSC2*.

At the completion of 12 weeks of treatment, hypomelanotic macules on non-sun-exposed and sun-exposed skin improved clinically (Figure 1), but significant differences in their conditions at the initiation of treatment were demonstrated only on sun-exposed skin. However, after the discontinuation of treatment, the hypomelanotic macules were more improved than they had been at the completion of treatment, and significant differences were observed at 4 weeks after discontinuation of treatment for non-sun-exposed and sun-exposed skin ($P < .05$ and $P < .01$, respectively) and 12 weeks ($P < .01$) after discontinuation of the treatment on both areas (Figure 2). These results suggest that topical rapamycin therapy was as effective for the hypomelanotic macules in TSC as it is for TSC angiofibromas. However, recovery of the skin color took longer than recovery after angiofibromas, and once skin color was recovered, the effect lasted for several weeks after the discontinuation of treatment. Although topical rapamycin was effective in hypomelanotic macules and angiofibromas, the mechanisms by which the rapamycin improved the hypopigmented macules compared with the angiofibromas might be different.

To examine how rapamycin was involved in the melanogenesis, histochemical and electron microscopic examinations of the hypomelanotic macules in patients with TSC were conducted. Skin color is related to the number of melanin granules in keratinocytes that are transferred from melanocytes.[31] Our observations revealed that the melanosomal transfer from melanocytes to keratinocytes was normal (Figure 3A). Therefore, hypomelanotic macules in patients with TSC could be attributed to abnormal melanization in melanocytes but not to the abnormal transfer of melanosomes from melanocytes to keratinocytes or to the increased degradation of melano-

somes in keratinocytes, as reported by Murase et al.[21] We demonstrated that maturation of melanosomes was observed in the melanocytes of hypomelanotic macules in the patients with TSC (Figure 3A), but the numbers of matured melanosomes in the melanocytes varied (range, 5-63) between melanocytes (Table and eFigure 2 in the Supplement). Jimbow[32] reported that TSC-related hypomelanotic macules are associated with a decrease in melanization and melanosome size and an increase in aggregated melanosomes in keratinocytes. Our observations confirmed those findings. The abnormal melanization that resulted in variable numbers of melanosomes between melanocytes might be characteristic of the hypomelanotic macules in TSC. Rapamycin treatment reduced these abnormalities significantly, and the numbers of matured melanosomes in melanocytes after rapamycin treatment became uniform (range, 38-49) (Figure 3D). Because these posttreatment data are derived from a single patient, further studies will be necessary.

Recently, some studies indicated the involvement of mTOR in melanization.[17-20] If we consider these results, mTORC1 might be involved in melanogenesis, and rapamycin regulated the constitutively activated mTORC1 and improved abnormal melanogenesis in the TSC melanocytes. Then, the gradual improvement of skin color was likely owing to the rapamycin treatment. Skin color further improved after discontinuation of the treatment.

To corroborate the effects of rapamycin on the melanocytes in the patients with TSC, TSC-model melanocytes before and after rapamycin treatment were observed with an electron microscope (Figure 4). The TSC-model melanocytes had significantly small numbers of melanosomes. The clinical findings in the pretreatment biopsy samples showed a variable number of melanosomes. However, TSC model melanocytes showed reduced numbers of melanosomes. We attribute this discrepancy to influence on melanocytes in vivo by many other surrounding cells and the intricate effect of the microenvironment, in contrast to cultured cells. In addition, *TSC2* may be inactivated by a mutation or a deletion, and these modes may not be functionally identical.[33] Some abnormality might induce the variability and another might induce the decrease of melanosomes. In patient 2, *TSC2* abnormality may have induced the decrease of melanosomes rather than the variability (eFigure 2 in the Supplement). Rapamycin treatment significantly increased the numbers of melanosomes in the TSC-model melanocytes, although no influence appeared in the control melanocytes (Figure 4). These results were consistent with the clinical symptoms after topical rapamycin treatment; namely, rapamycin treatment affected only the hypomelanotic macule but did not affect normal skin.

Although clinical improvement of hypomelanotic macules in sun-exposed skin was remarkable compared with the non-sun-exposed skin throughout the study period, including at initiation (Figure 1), the difference in δ-L values between the sun-exposed skin and the non-sun-exposed skin was not statistically significant (Figure 2C). Recently, Kalie et al[34] reported that ULK1 regulates melanin levels in MNT-1 melanoma cells independently of mTORC1. Sun exposure might improve melanogenesis in an mTOR-independent manner.

In this trial, no adverse effects were observed, and the rapamycin blood concentrations were lower than the detection limit (0.6 ng/mL) in all participants. Because mTORC1 is a conserved and ubiquitous protein, adverse effects are a concern with the systemic administration of rapamycin. Topical rapamycin treatment is recommended not only for facial angiofibromas but also for hypomelanotic macules attributable to TSC.

Conclusions

In this report, we clarified that topical rapamycin therapy was effective and safe for treating TSC-related hypomelanotic macules. We also revealed that the small or varied numbers of melanosomes in melanocytes might be characteristic of TSC-related hypomelanotic macules and that rapamycin improved the abnormalities. Sun exposure might improve melanogenesis in an mTOR-independent manner. Further studies with more samples are expected.

ARTICLE INFORMATION

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Two Japanese Cases of Birt-Hogg-Dubé Syndrome with Pulmonary Cysts, Fibrofolliculomas, and Renal Cell Carcinomas

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Key Words
Birt-Hogg-Dubé syndrome · Fibrofolliculoma · Pulmonary cyst · Renal cell carcinoma

Abstract
Birt-Hogg-Dubé syndrome (BHD) is a rare autosomal dominant inherited disease caused by a germline mutation in the *folliculin* gene mapped in the region of chromosome 17p11.2. BHD predisposes the patient to cutaneous fibrofolliculomas (FFs), pulmonary cysts (PCs), and renal cell carcinoma (RC). Here, we present two cases of BHD in Japanese patients. One patient was a 37-year-old female, and the other a 35-year-old male. Each of the patients was affected by all three symptoms of BHD. Both patients had unremarkable FFs, asymptomatic PCs, and asymptomatic RC. The presence of RC was revealed by abdominal ultrasonic examination. We also summarized the data from 62 Asian cases of BHD from the available literature and found that their FFs were unremarkable. In addition, the proportion of BHD patients with FF is smaller for Asian patients than it is for Caucasian patients. We also found that it is rare for BHD patients in Asia to show all three symptoms of BHD. Careful inspection of the skin as well as skin biopsies are important for the early detection of BHD cases in Asia.

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Case Presentations

Case 1

The first patient was a 37-year-old female who presented with an asymptomatic left renal tumor that was revealed by ultrasound and computed tomography (CT) examinations (fig. 1a). The CT examination also revealed the presence of asymptomatic bilateral multiple PCs (fig. 1e). Since the age of 35, a few firm papules had developed on her face and head (fig. 1g, h). The patient had no family history of pulmonary, renal, or cutaneous disease. A laparoscopic left nephrectomy was performed. On gross examination, the resected kidney was a yellowish, solid tumor with partial hemorrhage (fig. 1b). The tumor was identified as a clear cell renal carcinoma (fig. 1c) positive for vimentin stain (fig. 1d) and negative for CK-7. A skin biopsy of the papule on her head revealed it was a fibrofolliculoma (FF; fig. 1i, j) positive for factor 13a (fig. 1k) and c-kit (fig. 1l), and negative for CD34, α-smooth muscle actin (α-SMA), S100, and CD68. Because the findings for this patient met 1 major criterion and 2 minor criteria [1], she was diagnosed with Birt-Hogg-Dubé syndrome (BHD).

Case 2

The second patient was a 35-year-old male who presented with an asymptomatic right renal tumor. The presence of the tumor was revealed by ultrasound and CT examinations (fig. 2a). A laparoscopic right partial nephrectomy was performed, and the tumor was diagnosed as a chromophobe renal cell carcinoma (RC; fig. 2b). The carcinoma was negative for CD10, vimentin, and c-kit, and was partially positive for CK-7 (fig. 2c) and colloidal iron (fig. 2d). When the patient was 40 years of age, a CT examination revealed the presence of asymptomatic bilateral multiple PCs (fig. 2e). His mother, aunt, uncle, and grandfather all had histories of recurrent pneumothorax (fig. 2f). A few firm papules were also present on the patient's face (fig. 2g). The results of the skin biopsies revealed that the papules were FFs (fig. 2h, i) positive for factor 13a (fig. 2j) and c-kit (fig. 2k), and negative for CD34, α-SMA, S100, and CD68. Because the findings for this patient met 1 major criterion and 2 minor criteria [1], he was diagnosed with BHD.

Discussion

BHD is a rare autosomal dominant inherited disease that is caused by a germline mutation in the *folliculin* gene mapped in the region of chromosome 17p11.2. This gene is involved in the signaling of the mammalian target of rapamycin [1]. In cases from Asia, the mutations are located on exons 5, 6, 9, 11, 12, 13, and 14, and on intron 5 (table 1) [2, 3]. The disease is characterized by the presence of cutaneous FFs, multiple PCs, and RC.

Toro et al. [4] reported that FFs are present in 90% of the families with BHD. The skin lesions usually appear as multiple, dome-shaped, whitish papules on the face after the age of 20 years. They are hamartomas that originate from the hair follicle, and are positive for CD34, factor 13a, c-kit, and CD68, and negative for α-SMA. The FFs in both of our cases were positive for factor 13a and c-kit, and were negative for α-SMA, CD34, and CD68.

Menko et al. [1] reported that PCs are present in more than 80% of the adult patients with BHD. These PCs are most often located as basal lung lesions. The median age at first occurrence is 38 years, and the pathological findings of the lung are not so specific. In case 1, there was a layer of alveolar epithelial cells on the inner wall of the PCs.

Pavlovich et al. [5] reported that renal tumors are present in 27% of the BHD patients at a mean age of 50.4 years. Histologically, they are hybrid oncocytic tumors in 67%, chromo-

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phobe RCs in 23%, and clear cell RCs in 7% of the BHD patients. Chromophobe RCs are positive for CK7, c-kit, and colloidal iron, partially positive for CD10, and negative for vimentin. Case 2 was diagnosed with chromophobe RC by hematoxylin-eosin stain, which was positive for CK7, partially positive for colloidal iron and negative for vimentin, CD10, and c-kit. Clear cell RC was positive for vimentin and partially positive for CK-7. Case 1 was diagnosed with clear cell RC by hematoxylin-eosin stain, which was positive for vimentin but negative for CK7.

More than 60 cases of BHD in Asia have been reported in the BHD literature. Kunogi et al. [2] described 30 BHD patients with pneumothorax and/or PCs and an *FCLN* mutation. Six (20%) cases had cutaneous lesions, only 1 (3%) of these was histologically diagnosed as FF, and only 1 patient (3%) had RC (table 2). Furuya and Nakatani [3] described 45 patients from 19 Asian families. Thirteen (29%) had FFs, 40 (89%) had PCs, and 9 (20%) had RCs. PCs, RCs, and FFs were present in only 2 of the patients (4%) (table 2).

We summarized 62 Asian case reports of BHD, not including those by Kunogi et al. [2] and Furuya and Nakatani [3] (table 1) [6–14]. Seventeen (27%) patients had FFs or perifollicular fibromas (PFFs) [6–10], 46 (79%) had PCs [10–13], 11 (18%) had RCs (aged 43–69 years, except for our cases; table 2) [8, 12, 14], 5 (8%) had PCs and FFs or PFFs [10], 5 (8%) had PCs and RC [12], and 2 (3%) had RCs and FFs or PFFs [8]. Only 2 cases had all three symptoms of PCs, RCs, and FFs or PFFs [3]. Therefore, our cases are only the third and fourth reported Asian BHD cases affected by all three symptoms. Compared with the other cases, our patients were younger (37 and 35 years) and had quiet FFs on the face and scalp lesions that were unremarkable. Shin et al. [10] described a Korean case with quiet FFs that appeared to be sebaceous hyperplasia. Kim et al. [12] also described a Korean BHD patient with quiet papular lesions that were 0.5–5 mm in diameter, but they were not diagnosed by biopsy.

Tsai et al. [15] reported that the Chinese scalp contains 0.72 follicular units (FUs) and the Caucasian scalp contains 1 FU/mm^2 surface area. The average hair density is 1.37 mm^2 in Chinese and 2 mm^2 in Caucasians. Total hair count and the number of FUs in Asians are smaller than in Caucasians. This difference may account for the few and unremarkable FFs present on the faces and scalps of Chinese, Japanese, and Korean patients.

Careful inspection of the skin and skin biopsies are important for the early detection of Asian BHD. Early detection will lead to the early diagnosis of RC and reduce BHD mortality.

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Table 1. Asian cases of BHD except Kunogi et al. [2] and Furuya and Nakatani [3]

Case	Year	First author	Age, years	Gender	Skin histology	PC	Family history	RC	Mutation
1	1990	Ishiko [16]	46	M	ff, td, ac	–	–	–	UK
2	2000	Aoki [6]	65	M	ff, td	–	+s	–	UK
3	2001	Takahashi [17]	53	F	ff	–	+ff	+	UK
4	2003	Ishihara [18]	52	F	ff, td, ac	–	+s		UK
5	2004	Kawasaki [7]	26	M	ff	–	+s	–	E11
6			UK	M	NT	–	fath. of 5	–	E11
7	2005	Nagashima [19]	51	F	NF	–	–	+	NF
8	2007	Murakami T [14]	55	UK	NF	–	–	+	E11
9	2007	Gunji [11]	30	F	NF	+	+p	–	E13
10			38	F	NF	+	+p	–	E11
11			40	F	NF	+	+p, r	–	E6
12			37	F	NF	+	+p, r	–	I5
13			38	F	NF	+	+p, r	–	E12
14	2007	Saito [20]	35 (Ch)	M	pff	–	–	–	UK
15	2008	Sadamasa [21]	35	M	ff, td	+	+s	–	UK
16	2008	Misago [22]	67	F	ff, td	–	+s	–	E13
17			38	F	not ff, td	+	dau. of 16	–	E13
18			35	F	not ff, td	+	dau. of 16	–	E13
19	2008	Kim [23]	31 (Ko)	F	pff, td	+	+p	–	E14
20	2009	Imada [8]	68	M	ff	–	+s, r	+	E12
21	2009	Miyasaka [24]	34	F	trunk, NT	+	+p	–	E12
22	2009	Ando [25]	58	F	ff, ac?	+	+p	–	E12
23	2009	Ishii [26]	29	F	NF	+	+p	–	UK
24			40	M	NF	+	bro. of 23	–	E6
25			72	M	NF	+	fath. of 23	–	UK
26	2009	Koga [27]	41	F	NF	+	+ff	–	E12
27			UK	M	ff	–	fath. of 26	–	E12
28	2009	So [28]	57 (Ch)	F	ff	+	+ p	–	E13?
29			31	F	NF	+	dau. of 28	–	E13?
30			28	F	NF	+	dau. of 28	–	E13?
31	2010	Hayashi [29]	39	F	NF	+	–	–	E13
32	2011	Nakagawa [30]	30'	F	NF	+	+p, r	–	E12
33			30'	M	NF	+	bro. of 32	–	UK
34	2011	Sakairi [31]	41	F	NF	+	+ff	–	E12
35			55	F	NF	+	+p	–	E12
36	2011	Park [9]	43 (Ko)	M	ff, td	–	+s, p	–	E11
37	2011	Shin [10]	46 (Ko)	F	NF	+	dau. of 37	+	E5
38			69	F	NF	+	+p, r	+	E5
39	2012	Nagashima [32]	69	F	NF	+	–	+	E5
40	2012	Ishizuka [33]	45	F	NT	+	+p	–	E11
41	2012	Kim [12]	40 (Ko)	F	not ff	+	+p, r	–	E6
42			UK	M	UK	+	fath. of 41	+	UK
43			UK	F	NF	+	sis. of 41	–	E6
44			UK	F	NF	+	sis. of 41	–	E6
45	2012	Kashiwada [34]	60	F	NT?	+	+s, p	–	E11

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46			32	M	NT?	+	son of 39	–	E11?
47	2012	Tobino [35]	43	F	NF	+	+p	+*	E12
48			UK	M	NF	+	son of 47	–	UK
49	2013	Ema [36]	53	F	not ff	+	–	+	E6
50	2013	Nishii [13]	33	F	not ff	+	+s, p	–	I5
51			UK	M	extrm., NT	+	fath. of 50	–	I5?
52			UK	M	UK	+	uncle of 50	–	UK
53			UK	F	UK	+	G.M. of 50	–	UK
54	2013	Haga [37]	61	F	NF	+	+p	–	E11
55			29	M	NF	+	son of 54	–	E11
56			UK	M	NF	+	bro. of 54	–	UK
57	This report	Murakami Y	37	F	ff	+	NF	+	UK
58			35	M	ff	+	+p	+	UK
59			UK	F	UK	+	moth. of 58	–	UK
60			UK	F	UK	+	aunt of 58	–	UK
61			UK	M	UK	+	uncle of 58	–	UK
62			UK	M	UK	+	G.F. of 58	–	UK

Ch = Chinese; Ko = Korean; UK = unknown; ff = fibrofolliculoma; td = trichodiscoma; ac = acrochordon; NF = no cutaneous change could be found; NT = not tested; extrm. = extremity; s = skin lesion; p = pulmonary lesion; r = renal lesion; dau. = daughter; sis. = sister; bro. = brother; fath. = father; G.M. = grandmother; moth. = mother; G.F. = grandfather; E = exon; I = intron.

Diagnosed as renal cyst by abdominal sonography without pathological examination.

* Diagnosed as angiomyolipoma by CT images without pathological examination.

Table 2. Clinical findings of Asian cases compared with those in the USA or Europe

	Toro [4], Menko [1], Pavlovich [5]	Kunogi [2]	Furuya [3]	Murakami Y, this report
Fibrofolliculoma	90%	20%*	29%	27%
Pulmonary cyst	80%	100%	89%	79%
Renal cell carcinoma	27%	3%	20%	18%

* Only 3% of the patients had a pathological diagnosis.

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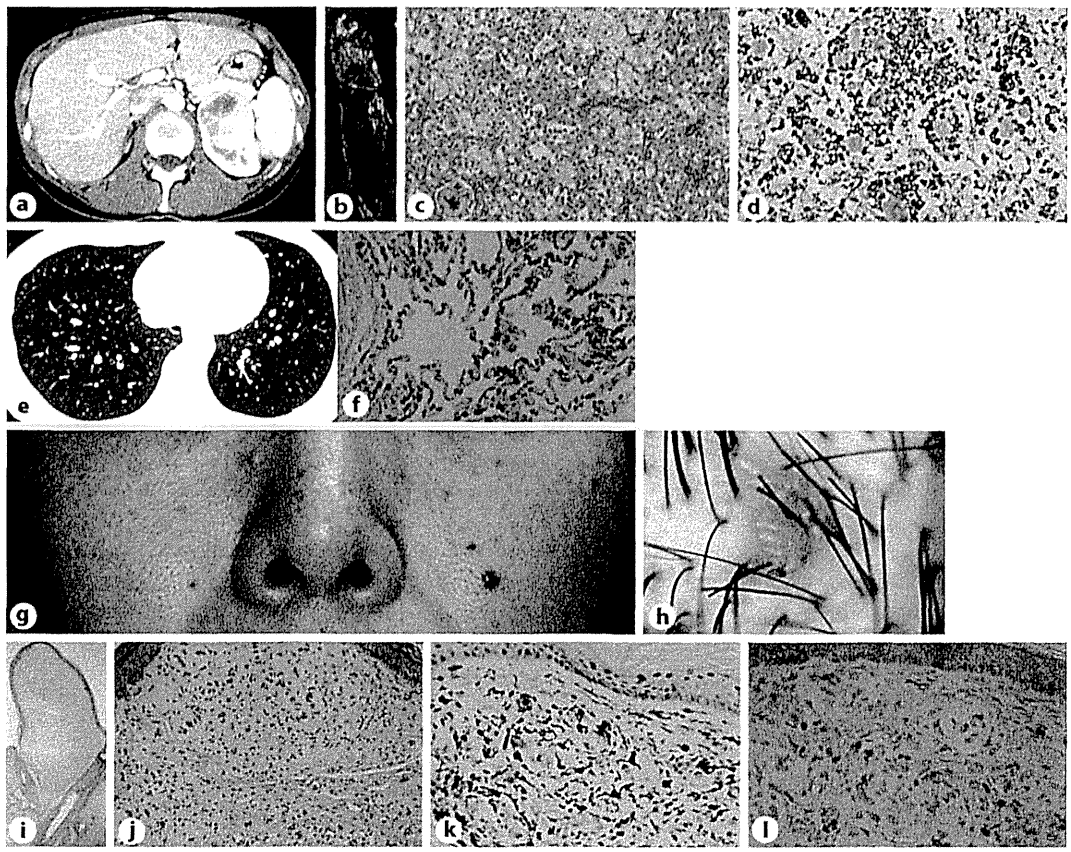

Fig. 1. The abdominal CT revealed a low-density area on the left kidney of a 37-year-old female with BHD (**a**), which was histopathologically diagnosed as clear cell carcinoma. On gross examination, the resected kidney contained a partially hemorrhagic, yellowish, solid tumor (**b**). The tumor was composed of cells with clear to acidophilic cytoplasm (hematoxylin-eosin stain; **c**), which were positive for vimentin stain (**d**; 200× magnification). The thoracic CT revealed bilateral, multiple PCs in the basilar and mediastinal regions (**e**). Microscopic findings for the lung revealed that the septum of the cyst wall contained capillaries and was lined by pneumocytes on both surfaces (**f**; 200× magnification). Quiet, skin-colored papules were present on the patient's face (**g**). There were few pinkish-colored, verrucous papules on the scalp (**h**). Histopathological images (hematoxylin-eosin) of the FF. The stoma was rich in fibroblasts and was oriented in parallel bundles of root sheath-like fibers and neighboring enlarged hair follicles [20× magnification (**i**) and 200× magnification (**j**)] positive for factor 13a (**k**) and c-kit (**l**; 400× magnification).

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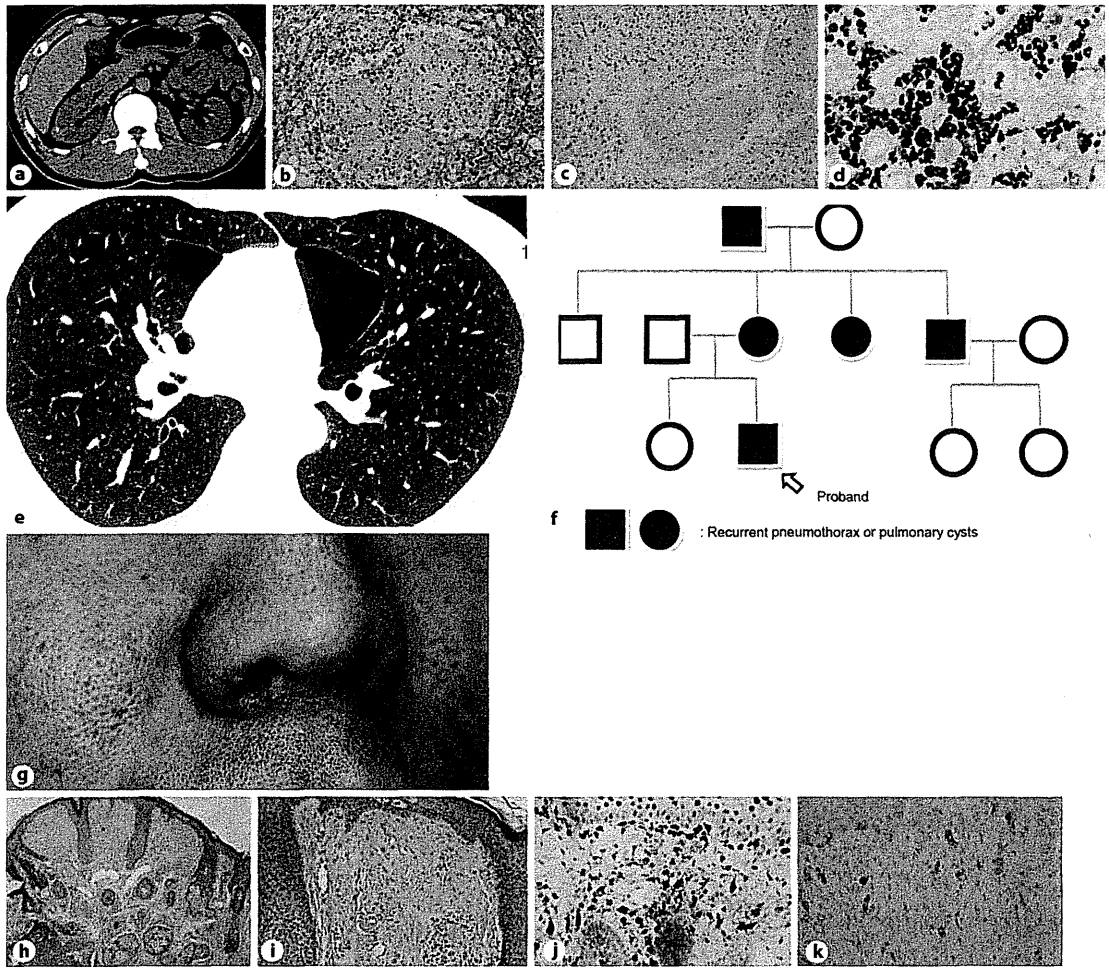

Fig. 2. The abdominal CT revealed a low-density area on the right kidney of a 35-year-old male with BHD (**a**). The tumor was composed of cells with eosinophilic cytoplasm (hematoxylin-eosin; **b**), was partially positive for CK7 (**c**; 200× magnification) and colloidal iron (**d**; 400× magnification), and histopathologically diagnosed as chromophobe cell carcinoma. A thoracic CT revealed the presence of bilateral, basally located, multiple PCs (**e**). Pedigrees of the families affected with recurrent pneumothorax (**f**). Skin-colored papules were present on the nose (**g**). Histopathological images (hematoxylin-eosin) of the FFs [40× magnification (**h**) and 200× magnification (**i**)] positive for factor 13a (**j**) and c-kit (**k**; 400× magnification).

Consensus Statement

Dermatologic and Dental Aspects of the 2012 International Tuberous Sclerosis Complex Consensus Statements

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Editor's Note

IMPORTANCE The 2012 International Tuberous Sclerosis Complex Clinical Consensus Conference was convened to update the last consensus statement in 1998. Skin and dental lesions are common in tuberous sclerosis complex (TSC) and are a frequent concern for patients. Recognition of these lesions is imperative for early diagnosis, given the treatment advances that may improve patient outcomes.

OBJECTIVE To detail recommendations for the diagnosis, surveillance, and management of skin and dental lesions in TSC.

EVIDENCE REVIEW The TSC Dermatology and Dentistry Subcommittee, 1 of 12 subcommittees, reviewed the relevant literature from 1997 to 2012.

FINDINGS A consensus on skin and dental issues was achieved within the Dermatology and Dentistry Subcommittee before recommendations were presented, discussed, and agreed on in a group meeting of all subcommittees from June 14 to 15, 2012.

CONCLUSIONS AND RELEVANCE Skin and dental findings comprise 4 of 11 major features and 3 of 6 minor features in the diagnostic criteria. A definite diagnosis of TSC is defined as the presence of at least 2 major features or 1 major and 2 or more minor features; in addition, a pathological mutation in *TSC1* or *TSC2* is diagnostic. Skin and oral examinations should be performed annually and every 3 to 6 months, respectively. Intervention may be indicated for TSC skin or oral lesions that are bleeding, symptomatic, disfiguring, or negatively affecting function. Options presented include surgical excision, laser(s), or use of a mammalian target of rapamycin inhibitor.

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Tuberous sclerosis complex (TSC) is a genetic disorder variably manifesting as cognitive impairment, seizures, and hamartomas of the brain, eyes, kidneys, heart, lungs, and skin.[1] The diagnosis and management of TSC has evolved since the time of the previous international TSC consensus meeting in 1998. The spectrum of clinical manifestations has been refined, aided by the ability to better define cohorts of patients by testing for mutations in the causative genes *TSC1* and *TSC2*.[2] Treatment of patients has been transformed by the use of mammalian target of rapamycin (mTOR) inhibitors, drugs that target the signaling pathway activated in TSC tumors as a consequence of loss of function of the TSC1-TSC2 protein complex.[3-6] As a result of these advances, the Tuberous Sclerosis Alliance convened an international group of clinical experts and scientists to update the consensus documents of 1998.[7,8] The goal of this article was to provide additional detail of the dermatological and dental aspects of the updated diagnostic criteria[9] and management recommendations.[10]

Methods

A subcommittee on dermatology and dentistry, 1 of 12 subcommittees, was composed of 7 dermatologists and 2 dentists with expertise in TSC. The subcommittee reviewed the relevant literature and arrived at a consensus opinion regarding dermatological and dental diagnostic criteria and treatment of TSC. These recommendations were presented to the entire group of 79 specialists from 14 countries for discussion and approval at the meeting in Washington, DC, from June 14 to 15, 2012. Final recommendations were incorporated into manuscripts reporting the updated diagnostic criteria[9] and guidance for surveillance and treatment of TSC.[10] In anticipation of more detailed recommendations that would be published in disease focus areas,[10] the Dermatology and Dentistry Subcommittee detailed these recommendations in the present article, supported with updated literature since the last consensus statement. Patients who provided consent for photographs were

Table 1. Revised Diagnostic Criteria for Tuberous Sclerosis Complex[a]

1998	2012
Genetic Criterion	
None	**Pathogenic mutation in *TSC1* or *TSC2***
Major Features	
Facial angiofibromas or forehead plaque	Angiofibromas (≥3) or **fibrous cephalic plaque**
Hypomelanotic macules (≥3)	Hypomelanotic macules (≥3, **at least 5 mm in diameter**)
Nontraumatic ungual or periungual fibroma	Ungual fibromas (≥2)
Shagreen patch (connective tissue nevus)	Shagreen patch
Multiple retinal hamartomas	Multiple retinal hamartomas
Cortical tuber	Cortical **dysplasias**
Subependymal nodule	Subependymal nodules
Subependymal giant cell astrocytoma	Subependymal giant cell astrocytoma
Cardiac rhabdomyoma, single or multiple	Cardiac rhabdomyoma
Lymphangioleiomyomatosis	Lymphangioleiomyomatosis[b]
Renal angiomyolipoma	Angiomyolipomas (≥2)[b]
Minor Features	
Multiple randomly distributed pits in dental enamel	Dental enamel pits (≥3)
Gingival fibromas	**Intraoral** fibromas (≥2)
"Confetti" skin lesions	"Confetti" skin lesions
Nonrenal hamartomas	Nonrenal hamartomas
Multiple renal cysts	Multiple renal cysts
Retinal achromic patch	Retinal achromic patch
Hamartomatous rectal polyps	
Bone cysts	
Cerebral white matter migration lines	

[a] Adapted from Roach et al[7] and Northrup and Krueger.[9] Items in boldface indicate changes from the prior criteria.

[b] A combination of lymphangioleiomyomatosis and angiomyolipomas without other features does not meet criteria for a definite diagnosis.

enrolled in protocol 00-H-0051, approved by the National Heart, Lung, and Blood Institute Institutional Review Board.

Results

Diagnostic Criteria

Perhaps the most significant change in the diagnostic criteria is the addition of a genetic criterion (**Table 1**). The demonstration of a pathogenic mutation in *TSC1* or *TSC2* in normal tissue is now considered sufficient for diagnosis, independent of clinical manifestations.[9] The use of DNA testing as an independent criterion may facilitate early diagnosis of affected individuals who have not yet manifested sufficient clinical diagnostic features.

Clinical diagnostic criteria continue to be important in diagnosis because genetic testing may not identify a mutation in up to 25% of patients with TSC and a normal gene test result does not exclude TSC.[2] The updated clinical criteria are still divided into major and minor features (Table 1). A definite diagnosis is defined as the presence of at least 2 major features or 1 major and 2 or more minor features. The diagnosis of TSC is considered possible in the presence of 1 major or 2 or more minor features.[9]

The updated clinical criteria include a few noteworthy changes to the extracutaneous criteria. Cortical dysplasia replaces cortical tubers as a major feature to encompass both cortical tubers and cerebral white matter radial migration lines. While angiomyolipomas remain a major feature, anatomic location is no longer limited to the kidney and may include the liver or other organ systems. Hamartomatous rectal polyps and bone cysts were deleted as minor criteria due to lack of specificity.[9]

Dermatologic and Dental Criteria

The dermatologic and dental lesions used in the 1998 consensus are maintained in the updated criteria,[9] including hypomelanotic macules, angiofibromas, ungual fibromas, shagreen patch, "confetti" skin lesions, and dental pits (Table 1). These lesions are usually readily identified based on their appearance (**Figure**).[11] The updated criteria incorporate several changes in terminology or number. The term *forehead plaque* is replaced with *fibrous cephalic plaque* because similar plaques may occur elsewhere on the face and scalp in patients with TSC. Similarly, oral fibromas are frequently gingival in TSC, but they may be observed at other intraoral sites including the buccal and labial mucosa as well as the tongue.[12] Therefore, the minor criterion of gingival fibromas is expanded to intraoral fibromas. *Ungual fibroma* is now recommended as a general term that encompasses periungual and subungual fibromas.[9]

In the 1998 criteria, only hypomelanotic macules had a numerical requirement (≥3). In the updated criteria, numerical requirements are added for several lesions, including angiofibromas (≥3), ungual fibromas (≥2), dental pits (≥3), and intraoral fibromas (≥2). The updated criteria also specify that hypomelanotic macules measure 5 mm or greater in largest diameter. Areas of poliosis may also be included in the count of hypomelanotic macules. Additional details about the rationale for these changes appear in the recent consensus document,[9] and herein we present recommendations of the dermatology and dental group regarding the application of these criteria and the presence of other TSC dermatological and dental lesions.

Application of the Dermatologic and Dental Criteria

Age Dependence

Tuberous sclerosis complex is a disease in which each lesion has a typical age of onset and periods for progression, stabilization, and in some cases, spontaneous resolution.[13,14] Hypomelanotic macules frequently present within the first few years of life, remain stable for decades, and become less apparent in late adulthood. Angiofibromas often start to appear at age 3 to 4 years, increase in number and size throughout the teenage years, and become relatively stable in extent throughout adulthood. Ungual fibromas have the most tardive onset of cutaneous manifestations, as they typically arise during adolescence or occasionally adulthood.[14] In light of the age-related penetrance of skin lesions, clinicians should not discount the possibility of TSC based on the absence of skin lesions, especially in infants. Guidance to parents to seek medical evaluation if new lesions appear may be beneficial. A correct understanding of the natural history also helps prevent lesion misidentification. For example, hypopigmented macules that are growing out of proportion to the child's growth or acquired in adolescence or adulthood