Buruli ulcer (BU) is a severe skin infectious disease caused by Mycobacterium ulcerans. It usually manifests as an indurated plaque that develops extensive necrosis of the affected skin. Mycobacterium ulcerans is considered an environmental pathogen, residing in an aquatic locale, and is thought to be transmitted by direct contact with contaminated water through minor trauma or by uncertain aquatic vectors that bite humans. Buruli ulcer most frequently occurs in tropical and subtropical areas, especially in West African countries such as Uganda, Ghana, and Benin. Other known endemic areas are located in Australia, Southeast Asia, China, Central America, and South America. According to a study by the World Health Organization, more than 5000 cases are reported annually from more than 30 countries; however, many unreported cases of BU are presumed to exist.

In 1982, Mikoshiba et al described a Japanese patient seen with an ulcerative lesion simulating BU. The isolated Mycobacterium from the lesion had bacteriological characteristics similar to those of M ulcerans, and the strain was later named M ulcerans subsp shinshuense based on the homology of the 16S ribosomal RNA (rRNA) gene sequence and the presence of insertion sequence 2404 (IS2404), which are specific to M ulcerans and related species. Since that first report, more than 30 Japanese patients diagnosed as having BU have been described in the literature. We report herein additional cases of BU that concurrently occurred in a Japanese family.

Report of Cases

Case 1
In December 2010, a 2-year-old Japanese girl from the Aizu region of the Fukushima prefecture presented with asymptomatic swelling of her right cheek. One month earlier, her family had first noticed the eruption, which had gradually enlarged. At first examination, an indurated plaque, 5 cm in diameter, with a central ulcer was observed on the right cheek (Figure 1A). The patient was afebrile and otherwise healthy. She was treated with oral cefcapene pivoxil for 1 week, without benefit. Based on a presumed diagnosis of nontuberculous mycobacterial in-
Infection or neoplastic disease, a skin biopsy specimen was obtained from the right cheek. A hematoxylin-eosin–stained section showed extensive necrosis of deep dermis and subcutaneous fat (Figure 1B). Coagulation of dermal vessels was also seen. Inflammatory infiltrates were observed only scarcely (hematoxylin-eosin, original magnification ×100). A slightly red indurated plaque with a small central ulcer on the left leg. Numerous acid-fast bacilli detected by Ziehl-Neelsen stain of the biopsy specimen of a leg lesion (original magnification ×400).

In late December 2010, an indurated plaque with a small ulcer appeared on her left leg (Figure 1C). A biopsy specimen from the leg lesion demonstrated numerous acid-fast bacilli by Ziehl-Neelsen stain.

Case 2
The 5-year-old brother of patient 1 subsequently was seen because of an asymptomatic indurated eruption that had appeared on the right forearm 3 weeks earlier, and the center of the lesion had gradually ulcerated. At first examination, a large plaque, 6 cm in diameter, was seen on the ulnar aspect of the right forearm (Figure 2A). His medical history was unremarkable. A skin biopsy specimen revealed the same histological findings as had been observed in patient 1. Acid-fast bacilli were detected in a smear and formalin-fixed section by Ziehl-Neelsen stain.

Case 3
A month later, the previously healthy 37-year-old mother of patients 1 and 2 was seen to discuss her children's conditions, she was noted to have an indurated red plaque, 4 cm in diameter, on her right wrist (Figure 2B). She had noticed the eruption around the time her children's condition was noticed,
which had gradually enlarged, without any subjective symp-
toms. A biopsy specimen revealed the same findings as had be-
been observed in patients 1 and 2.

**Clinical Course and Treatment**

All 3 patients were referred for further investigation and treat-
ment of their disease. According to a tentative diagnosis of BU
based on the histological findings and detection of acid-fast bacilli from skin samples, the patients were treated with oral
administration of levofloxacin (12 mg/kg), clarithromycin (16
mg/kg), and rifampicin (10 mg/kg). The necrotic tissue of their
lesions on the extremities was surgically removed and covered by a skin graft. The facial lesion in patient 1 gradually healed without surgical treatment. Oral antimycobacterial
drugs were discontinued 9 months after treatment began. At
27 months’ follow-up, their lesions had not recurred.

**Bacterial Culture and Identification of Pathogenic Organisms**

Skin biopsy samples obtained from the patients were inocu-
lated on 2% Ogawa medium and incubated at 27°C. After 41 to
58 days of culture, small yellow-white colonies were obtained
from each sample. The sequence of the 16S rRNA gene in the isolated strains from our patients was identical to that of
*M. ulcerans* subsp *shinshuense* American Type Culture Col-
lection 33728 but differed from that of *M. ulcerans* Agty99 at
positions 492, 1288, and 1449–1451, which are known to be use-
ful for differentiating *M. ulcerans* subsp *shinshuense* from *M. ulcerans* *Agy99*.7 Insertion se-
quency 2404 was detected from the strains by PCR. In addi-
tion, our strains lacked 1 of 8 genes encoding mycolactone on
the virulence plasmid pMUM001 by PCR.7 Based on these find-
ings, all strains obtained from our patients were determined
to be *M. ulcerans* subsp *shinshuense*.

**Detection of Pathogenic Organism From the Environment Near the Residence of the Cases**

The family lives in a house surrounded by rice fields in a rural
town. A stagnant agricultural water channel exists in the back-
yard, where the 2 children usually play. After obtaining in-
formed consent from the family, we collected water samples
at 2 different sites of the channel and 2 crayfish. Detection of
the pathogenic strain was performed using a highly sensitive method capable of detecting small amounts of DNA as de-
scribed previously.7,8 Briefly, DNA extracts from concentra-
ted water and homogenized crayfish were subjected to
whole-genome amplification PCR,8 and the amplified DNA was
subsequently analyzed by PCR using primers targeting IS2404.7

The expected 278-base pair (bp) product corresponding to
IS2404 was detected from one of the crayfish, and the se-
quence of the 278-bp product was identical to that of IS2404 of
*M. ulcerans*.

**Discussion**

Buruli ulcer initially manifests as a small red papule or subcu-
taneous nodule that gradually extends to the periphery. A le-
sion develops with a large ulcerative plaque covered by ne-
crotic tissue, resulting in scarring, contracture, and disability.
Despite its large size, BU is usually painless,9 which often leads to
delay in seeking medical care until the lesion reaches an ad-
vanced stage. Cytotoxic and immunosuppressive properties of
mycolactone produced by *M. ulcerans* are considered to have a
major role in the pathogenesis of BU.9 Coagulation of blood ves-
sels, which is frequently observed in the skin lesions, may also
account for extensive necrosis of the affected skin.5,9

In 2011, Nakana et al10 reviewed clinical, geographic, and
bacteriological features of 19 Japanese patients diagnosed as
having BU. All cases were sporadic, with the age of patients
ranging from 8 to 81 years. The cases were distributed across
various areas of Honshu, the main island of Japan; no en-
demic focus was observed. Most patients first noticed their le-
sions during the autumn or winter. In contrast to the en-
demic areas in which BU is caused by *M. ulcerans*, all isolated
organisms from Japanese patients were determined to be *M. ulcerans* subsp *shinshuense* by sequencing of the 16S rRNA gene. Despite the difference in the pathogenic strains, clinical fea-
tures are similar between the Japanese patients and those from
the endemic areas except for the occasional report of a pain-
ful lesion, which is more frequent among Japanese.8 Buruli ulcer
is known to occur mainly in regions near wetlands, such as
ponds, swamps, and slow-flowing or stagnated water; how-
ever, no Japanese patients have been reported to have direct
evidence of contact with an aquatic environment before the
onset of their skin lesion.10

We report herein a rare instance of familial occurrence of
BU in Japan, in which 3 family members developed BU. Di-
rect transmission of the pathogenic organism among the family
during their daily life seems unlikely because their lesions
appeared almost simultaneously. In the endemic areas, a fam-
ily history of BU is observed in 12% to 23% of patients mani-
festing the lesions.8,11–14 Similar routines and a common envi-
ronmental exposure may increase the risk of familial
occurrence of BU.11,12 However, case-control studies have dem-

### Table. 16S Ribosomal RNA Gene Sequences Differentiating *Mycobacterium ulcerans* and Related Species

<table>
<thead>
<tr>
<th>Organism</th>
<th>Positions of Differing Residuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 TGGGAAA</td>
<td>492 TAAGGCC ACCC-TTTG</td>
</tr>
<tr>
<td>Case 2 TGGGAAA</td>
<td>1288 TAAGGCC ACCC-TTTG</td>
</tr>
<tr>
<td>Case 3 TGGGAAA</td>
<td>1449-1451 TAAGGCC ACCC-TTTG</td>
</tr>
<tr>
<td><em>M. ulcerans</em> subsp <em>shinshuense</em> American Type Culture Collection 33728</td>
<td>TGGGAAA TAAGGCC ACCC-TTTG</td>
</tr>
<tr>
<td><em>M. ulcerans</em> Agty99</td>
<td>TGGCGAA TAAGGCC ACCC-TTTTGG</td>
</tr>
</tbody>
</table>

*Underlined letters indicate the residues that delineate *M. ulcerans* subsp *shinshuense* from *M. ulcerans*.
onstrated conflicting results about the familial relationship of BU: some investigators concluded that there was no significant difference in the family history of BU between cases and controls, whereas other researchers suggested that a familial history of BU was associated with an increased risk of BU. It is thought that host genetic factors may influence individual susceptibility to the development of BU after exposure to Mycobacterium ulcerans. Therefore, a genetic predisposition might explain why some members of the family described herein who had a similar risk of exposure to the pathogenic organism did not develop BU.

In the endemic areas, Mycobacterium ulcerans has been detected from diverse environmental samples, such as soil, sediment, water bugs, and mosquitoes. We detected IS2404 from the crayfish captured in a water channel surrounding the house of our patients. However, direct transmission from crayfish is unlikely because of the location of the patients’ lesions. Detection of IS2404 from crayfish indicates the possibility that, similar to endemic areas, aquatic environments act as a reservoir of the pathogenic strain in Japan. Like other Japanese patients, our patients developed skin lesions in the winter. Considering the latent period of Mycobacterium ulcerans, which is estimated to be several months or longer, it is conceivable that our patients were infected with the pathogen in the summer and developed skin lesions after an incubation period of several months. If such a latency is true, aquatic insects, such as mosquitoes and blackflies, which become active and bite humans during the summer, may be possible vectors in Japan. Further investigation is needed to clarify the transmission pathway to prevent this serious infectious disease.