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. BU 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 are observed only scarcely (hematoxylin-eosin, original magnification ×100). C, A slightly red indurated plaque with a small central ulcer on the left leg. D, Numerous acid-fast bacilli detected by Ziehl-Neelsen stain of the biopsy specimen of a leg lesion (original magnification ×400).

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 symptoms. A biopsy specimen revealed the same findings as had been observed in patients 1 and 2.

### Clinical Course and Treatment

All 3 patients were referred for further investigation and treatment 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 inoculated 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 Collection 33728 but differed from that of *M. ulcerans* Agy99 at positions 492, 1288, and 1449-1451, which are known to be useful for differentiating *M. ulcerans* subsp *shinshuense* from *M. ulcerans* and *Mycobacterium marinum* (Table). Insertion sequence 2404 was detected from the strains by PCR. In addition, our strains lacked 1 of 8 genes encoding mycolactone on the virulence plasmid pMUM001 by PCR. Based on these findings, 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 backyard, where the 2 children usually play. After obtaining informed 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 described previously. Briefly, DNA extracts from concentrated water and homogenized crayfish were subjected to whole-genome amplification PCR, and the amplified DNA was subsequently analyzed by PCR using primers targeting IS2404. The expected 278-base pair (bp) product corresponding to IS2404 was detected from one of the crayfish, and the sequence 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 subcutaneous nodule that gradually extends to the periphery. A lesion develops with a large ulcerative plaque covered by necrotic tissue, resulting in scarring, contracture, and disability. Despite its large size, BU is usually painless, which often leads to delay in seeking medical care until the lesion reaches an advanced stage. Cytotoxic and immunosuppressive properties of mycolactone produced by *M. ulcerans* are considered to have a major role in the pathogenesis of BU. Coagulation of blood vessels, which is frequently observed in the skin lesions, may also account for extensive necrosis of the affected skin.

In 2011, Nakagawa et al 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 endemic focus was observed. Most patients first noticed their lesions during the autumn or winter. In contrast to the endemic 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 features are similar between the Japanese patients and those from the endemic areas except for the occasional report of a painful lesion, which is more frequent among Japanese. Buruli ulcer is known to occur mainly in regions near wetlands, such as ponds, swamps, and slow-flowing or stagnated water; however, no Japanese patients have been reported to have direct evidence of contact with an aquatic environment before the onset of their skin lesion.

We report herein a rare instance of familial occurrence of BU in Japan, in which 3 family members developed BU. Direct 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 family history of BU is observed in 12% to 23% of patients manifesting the lesions. Similar routines and a common environmental exposure may increase the risk of familial occurrence of BU. 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 Residue*</th>
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<tr>
<td></td>
<td>492</td>
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<tr>
<td>Case 1</td>
<td>TGGGAA</td>
</tr>
<tr>
<td>Case 2</td>
<td>TGGGAA</td>
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<tr>
<td>Case 3</td>
<td>TGGGAA</td>
</tr>
<tr>
<td><em>M. ulcerans</em> subsp <em>shinshuense</em> American Type Culture Collection 33728</td>
<td>TGGGAA</td>
</tr>
<tr>
<td><em>M. ulcerans</em> Agy99</td>
<td>TGGCAAA</td>
</tr>
</tbody>
</table>

*a Underlined letters indicate the residues that delineate *M. ulcerans* subsp *shinshuense* from *M. ulcerans*. Copyright 2014 American Medical Association. All rights reserved.
Buruli Ulcer Caused by Mycobacterium ulcerans

Case Report/Case Series Research

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Analysis and interpretation of data: All authors.
Drafting of the manuscript: Ohtsuka, Kikuchi, Yamamoto, Suzuki, Ishii.
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REFERENCES