2U DNase I (TaKaRa, Kyoto Japan) at 37 °C for 1 hour for preventing contamination with genomic DNA.

4.4. Reverse transcription and polymerase chain reaction (RT-PCR)

Highly expressed coding genomic, pseudogenomic and non-coding genomic regions determined from our tiling array results [17] were chosen for RT-PCR analysis. Reverse transcription was performed using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The primer sequences are shown in the Table 1, and 5′-ACTAGCGGTATCGATCTGAC-3′ and 5′-GTGATGCGTTGGAATTCGG-3′ for Hsp70 (ML2496) to detect the presence of *M. leprae*. Touchdown PCR conditions were described previously [29].

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sport, Science and Technology of Japan (to K.S.) and by a Grant-in-Aid for Research on Emerging and Reemerging Infectious Diseases from the Ministry of Health, Labor, and Welfare of Japan (to N.I.). The authors thank M. Mishima and S. Aizawa for helpful discussions.

Table 1
Primer sequence designed and used for RT-PCR.

	PCR target	Product length	Forward primer (5′-3′)	Reverse primer (5′-3′)
Gene	ML1426	207	CTGATCAGGACGGTCGGTAT	TCTGGCTTCGGTCTCAGTCT
	ML0410	214	CAGATTGGAGCGCATTACCT	ATAATCACCTGCGGCATCTT
	ML0576	206	TGGCGTCTGGTGTCAATATC	CCAGCATAGTCGAGTCACAGG
	ML1309	247	GACCTTCGAGGATCTGTTCG	ATAGACGTCATCGAGCGACA
	ML0198	186	GAACTGTGAAGTGGTTCAACG	AGCGAACTCCAGTGGCTTG
	ML0574	205	TTGCTAGTACGGCAACCAGA	TGTTGAACAGGACGGTATGG
	ML0275	263	AAGAACACCGCTTGAGTTGG	ATGTTCGAGGAACGGTTACG
	ML2580	563	CATCAACACCATGAGCATCC	TCTACCTGGCGTTGCCTATC
	ML0050	194	TCATCGTCGGTCTTGGTGTA	CTCAGCCAAGAACGGAACTT
	ML2247	511	GGCACCTTCACCAACCTAGA	CCAACTTAGGATCCGCTTGA
	ML1582	420	AGCTTGGTCGGTATGGTGAC	TGCTTGTGTAGCATCGGAAC
	ML2135	367	GAACATCATCGTGTGGTTGC	CGATGACACCGACTATGTGG
	ML2532	212	GTAAGTCGACGCAGCCGTAG	TGATGCGCAGCCAGATTA
	ML0519	318	AACAGCTGTCGGCTCTGATT	CAAGCAGGTAGCCTTGGACT
	ML1394	334	GTACGGATCGAGGATGCACT	GTCGCTGCAGTAATCGGTAA
	ML0319	213	TGTCCAGCCTTCTCGAAGTT	GTCCATCACCAGTGACATCG
	ML2691	259	CAGCGAGCTGGTAACTGACA	CAGTTCGTTGAGCCACTTGA
	ML1736	133	CAACGCCATCACGTAGTCAC	CATCACCGTGTACACCAAGC
	ML2042	451	AAGTGGCGCGTAGTGTTCTT	AGGCTGACGTCTTGAGCAAT
	ML2494	408	ATCCGTTGTCCATCTTCGAC	CAACATCGGTGACTTGTTCG
	ML0804	169	CGCTCGTCTTCACTCATGC	AGGATCCGGAGCTGTTCTTC
Pseudogene	ML1999	229	GATGCTGACATCGGCTACG	GCATCAATGCGACCAAGTTA
	ML1283	234	ACCTACCGCTGTTGACCATC	TGTAGCCATTGAGCACTTCG
	ML1867	522	ACCATCGCCGTGATCTTATC	TGCAAGACTCTGGTCATTCG
	ML1746	220	GAAGGTCTCGGTGGTGTGTT	CAACCGACTCATGTTGAACG
	ML0476	465	TCGCAACTTCACTGATCGTC	GTCTGGCACCAATACCGAGT
	ML2492	578	GGCTGGTCTGATGGTATCGT	GCGCGACATATTCACAGAGA
	ML0434	275	CGTGTCGGTAGTCCTTCCAT	AGCGAATCAACTGGAACACC
	ML2102	486	GCACATATGGTGCACACCTC	CCATTGGCTACAGGATACGG
	ML0278	215	GGCTGTCCGAATCATATTGC	GAGTCCACACACAACGATGAA
	ML2180	275	GGAATAGGCTTGTTGCGTGT	ATCATCCGGCTAGGAGCTG
	ML0870	239	GCAGGAGGAACTGGATTCAA	GTCCGATGTCTCGATGTCCT
	ML0307	333	AGTACAGCGTAGCGGTCAGC	TGACTTCCTGTGGCAATGAG
	ML0043	199	GCATTCTCGAGACAGTGCAA	TGGCCATGTCATCACTAGGA
	ML0434	201	TGGAACACCTCGTCGTATGTGG	TATAAGTGGCACCGCCGAACTC
	ML0794	191	AAAGACGGAGACTACGATG	GTTTAGAAGGTTGGTCGTTG
	ML1049	186	GCCTGCTAATTCTTGTCGATG	TCAGCGTGGATCAGAACTC
	ML1476	223	CGCCAGTAATCGTGTTGTCTG	TTGCCGCTTCCAATCCATC
	ML1721	233	TGTGCTCAAGTTCTTCCGT	GAGTCAAGGCTGATAGAAGGT
	ML2043	200	TCAACATGGCGATCTGCATTC	TGCGTGACCTTACAACGCT
Non-coding region	946125	156	GCGCCAAGGTATGAAGAACA	TTAGCGGATCCTCCTCGTAG
	2551060	194	ACATTCGAGACCAGCTACCG	TTCCGCTTGGAGGATAATTG
	2664658	227	TGAGCTTGCCGATTACGATT	GCCATTGAACTGGCCATC
	2114596	225	AGCCACACTGCACTTCACAC	TTCGCTAGTGTTGTGTGTTGG
	2546884	202	TCAATATGGCTTCCTATGTTGC	GCTGCATTAATCCATGATTCG
	2134972	242	CGGAATCCTGTTGACGTGTT	CGGCGCTAACAACTATCCTC
	2307322	242	GGTTCACCGGAAGAGTTGG	CGCGACGACTAAGCCAGTAG
	2951327	247	GTCTGTCCTGCGTTCTTGGT	ATCTAGCTTCGGAGGCATCA
	321367	299	GCAGCAATGGATAGCTGACA	AATCGATGTTGGTGGTTGGT
	1973155	276	GACGCTGAAGATGGTCGATT	GACGCTGAAGATGGTCGATT
	39277	180	TGAAGGCGATATCGATGCAG	ATGGTGCAAGGGATAACATCAG
	238514	191	TGCCGATGATTACATCATCC	CATCGAGTCCAAGCTCAAC
	348457	202	TGGACTCGATGTTGAAGTG	TGCTTAGCTATGCAGTGAG
	1450993	224	TGCGCTAGAAGGTTGCCGTATG	TCAATGTGGCCGCACCTGAA
	1593211	191	CATCGAGTCCAAGCTCAAC	TGCCGATGATTACATCATCC
	2152288	241	CCGATATGTTCGGTAGTCGT	GCATCGATATCGCCTTCAG
	2858681	217	ATGTTGGTTGAGCTTGGAC	TTGCTTAGCTATGCAGTGAG

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doi: 10.1111/j.1346-8138.2009.00637.x

ORIGINAL ARTICLE

Evaluation of polymerase chain reaction-based detection of *Mycobacterium leprae* for the diagnosis of leprosy

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ABSTRACT

Because *Mycobacterium leprae* cannot be cultivated *in vitro*, laboratory diagnosis of leprosy is generally made by microscopic and histopathological examination. The objective of the present study was to evaluate the sensitivity and utility of polymerase chain reaction (PCR) to detect *M. leprae* in comparison with other conventional methods for diagnosis such as split skin smears, histopathology and serodiagnosis. PCR amplification of the *M. leprae*-specific 16S ribosomal RNA was compared to other methods. Samples included 37 multibacillary (MB) patients with a positive bacteriological index (BI), 32 newly diagnosed paucibacillary (PB) patients whose BI were negative and 30 plaque psoriasis patients not residing in leprosy endemic areas as controls. The sensitivity of PCR was 30 fg of *M. leprae* DNA, which is equivalent to the DNA from 8.3 bacilli. The detection rate in MB and PB were 100% and 50%, respectively; the specificity was 100%. Semiquantitative evaluation of PCR correlated well with BI, but not with the morphological index (MI) nor with the serum antibody against phenolic glycolipid-1 (PGL-1). PCR detection of *M. leprae* targeting 16S ribosomal RNA was specific and more sensitive than conventional methods, and can contribute to early and accurate diagnosis of leprosy.

Key words: leprosy, *Mycobacterium leprae*, polymerase chain reaction.

INTRODUCTION

Leprosy is a chronic infectious disease where delay in diagnosis and treatment can lead to deformities, disabilities and social stigma for the rest of a patient's life. Despite being one of the earliest bacteria identified under the microscope, *Mycobacterium leprae*, the causative pathogen for leprosy, cannot be cultivated *in vitro*. Therefore, classical bacteriological methods to identify pathogenic bacteria cannot be applied for the diagnosis of leprosy. The differential diagnosis of leprosy has been performed based on clinical criteria and the presence of acid-fast bacilli (AFB) from tissue smears or tissue sections stained by Ziehl–Neelsen or Fite–Faraco methods. Non-polymerase chain reaction (PCR)-based detection of *M. leprae* DNA requires at least 10^4 organisms/g tissue in order to obtain reliable results.[1] Therefore, these methods are not routinely used as a diagnostic tool to detect *M. leprae*, particularly in patients with an indeterminate type at the tuberculoid end of the leprosy spectrum where AFB are generally rare or virtually absent.[2] This situation makes definitive and differential diagnosis difficult, especially in cases of paucibacillary (PB) patients and patients being monitored for possible relapse after completing multidrug therapy (MDT) led by the World Health Organization (WHO).

In the last two decades, new molecular biology methods, PCR amplification, have been developed

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Received 16 January 2008; accepted 14 January 2009.

as reliable and sensitive diagnostic tools for the detection of pathogens for many infectious diseases including leprosy.[3,4] Several investigators have used PCR to amplify various genomic sequences of *M. leprae* in order to improve detection of low numbers of bacteria.[5–10] In Vietnam, PCR has also been applied for the diagnosis of some communicable diseases other than leprosy.[11–13] Ideally, sensitive and specific methods such as PCR provide a promising approach for early diagnosis and treatment, leading to possible reduction of permanent deformities and disabilities, and a reduced socioeconomic burden due to leprosy in endemic countries.

In this study, we evaluated the usefulness of PCR analysis by comparing with other conventional methods, such as slit-skin smear, histopathological study and phenolic glycolipid-1 (PGL-1)-based serodiagnosis.

METHODS

Patients

Sixty-nine leprosy patients attending the National Institute of Dermato-Venereology, Da Nang Dermatology Hospital and Quy Hoa Central Leprosy-Dermatology Hospital in Vietnam between February and October 2004 were included in this study. These patients were divided into two groups: (i) 37 multibacillary (MB) patients with distinctive histopathological lesions typical of leprosy and a positive bacteriological index (BI) either newly diagnosed or being treated with WHO's MDT regimen; and (ii) 32 PB patients having a negative BI but distinctive histopathological lesions before receiving MDT. The MDT regimen used in MB patients was a combination of 600 mg rifampicin monthly, 300 mg clofazimine monthly, 50 mg clofazimine daily and 100 mg dapsone daily for a total of 12 months. Average ages in each group were 32.5 years (range, 10–76) and 33.4 years (range, 11–76), respectively. Forty-seven of 69 patients were men (23 MB and 24 PB) and 22 were women (14 MB and eight PB). The male : female ratio in this research was similar to annually reported data in Vietnam (unpubl. data from National Institute of Dermato-Venereology, Hanoi, Vietnam, "Leprosy Elimination Program Report from 1995–2004", 2005 in Vietnamese). Thirty plaque psoriasis patients hospitalized in the National Institute of Dermato-Venereology during the same period were selected as a control group, of whom 21 were men and nine were women with an average age of 51.3 years (range, 15–81). The study was approved by the Ethics Committee of the National Institute of Dermato-Venereology. Each patient signed a written informed consent prior to specimen collection.

Slit-skin smear and biopsies

Slit-skin smears and 4-mm punch biopsies were obtained from two adjacent positions on the border of the most active lesion found in each patient according to standard procedures. Slit-skin smears were also taken from both ear lobes. All smears were prepared on microscopic slides, stained by the classic Ziehl–Neelsen method and observed by well-trained technicians to identify AFB. BI and morphological index (MI) were evaluated according to Ridley's logarithmic scale.[14] BI of a patient is the mean of BI at all skin smear samples in his/her body. MI is the percentage of solid bacilli in the samples. Each biopsy sample was divided into two equal parts: one half was fixed in 4% (v/v) buffered neutral formalin (Sigma, Saint Louis, MO, USA) and then dehydrated in a graded series of ethanol (Sigma) and embedded in paraffin. Sections were stained by two methods: hematoxylin–eosin staining for histopathological examination and Fite–Faraco staining for the detection of AFB. The other half of the biopsy sample was frozen at –80°C for use in the PCR study. The results of skin smears and histopathological evaluations were used as criteria for grouping the patients as described above.

Serological examination of anti-PGL-1 antibody

A 5-mL venous blood sample was collected by venipuncture from each patient. Samples were centrifuged and serum was separated and kept frozen at –40°C until processed. Serum samples were tested for the presence of immunoglobulin (Ig)M anti-PGL-1 antibody using a *M. leprae* Particle Agglutination (MLPA) kit (Fujirebio, Tokyo, Japan) according to the manufacturer's procedures as described.[15] Briefly, three drops (75 μL) of serum diluent were added into the first well and one drop (25 μL) of the same diluent was added to the second and the third well of a 96-well U-type microdiluent plate. Test serum (25 μL) was added to the first well then mixed by pipetting, and the same volume of

diluted serum was serially transferred from the first to the second well and from the second to the third well. Diluted serum (25 mL) in the third well was discarded. One drop of unsensitized (without antigen) control particles and one drop of sensitized (antigen-bound) particles were then added to the second and the third wells, respectively. After being mixed briefly with the microplate mixer, the plate was incubated at 37°C for 1 h. The test was interpreted with the naked eye as negative if the sensitized particles formed a definite compact button with a smooth round outer margin. The result was regarded as positive if these particles formed one of the following: (i) a compact ring with smooth round outer margin; (ii) a significantly large ring with rough outer margin with agglutination in the periphery; or (iii) a filmy mat of homogeneous agglutination covering the entire bottom of the well.

DNA extraction

The frozen part of each skin biopsy specimen was incised into small pieces with sterile scissors and placed in a 1.5-mL sterile centrifuge tube containing 360 μL TE buffer (10 mmol/M Tris-Cl, pH 7.5 and 1 mmol/L ethylenediaminetetraacetic acid) (Bio-Rad, Hercules, CA, USA). Forty microliters of proteinase K (10 mg/mL) (Qiagen, Hilden, Germany) was added to the tube. The mixture was covered with mineral oil to prevent evaporation, incubated at 55°C for 18 h and at 97°C for 10 min to inactivate proteinase K. DNA was then extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) mixture (Bio-Rad). After separation by centrifugation for 5 min at 12 000 *g*, the DNA in the upper aqueous phase was precipitated with absolute ethanol and sodium acetate (Sigma). The DNA pellet was washed twice with 70% ethanol (Sigma), dissolved in 100 μL of TE buffer (Bio-Rad) and stored at −20°C until use. Purified *M. leprae* DNA as a positive control, obtained from experimentally infected mouse foot pads, purified *Mycobacterium smegmatis* DNA and purified *Escherichia coli* DNA were kindly provided by Dr Yasuko Yogi, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan.

PCR amplification

Primers P1, P2 and P3 employed in this study were previously published.[16,17] Primers P1 (5′-AGA GTT TGA TCC TGG CTC AG-3′) and P2 (5′-CGG AAA GGT CTC TAA AAA ATC TT-3′) served as forward primers whereas primer P3 (5′-CAT CCT GCA CCG CAA AAA GCT T-3′) was a reverse primer. These primers were synthesized by Invitrogen (Tokyo, Japan). P1–P3 primers amplify a 231-bp fragment of 16S ribosomal RNA coding gene of all Mycobacterium species,[18] while P2–P3 primers amplify a 172-bp fragment of the same gene, but more specific for *M. leprae* because primer P2 was designed to be a unique nucleotide sequence among these species. DNA solution (5 μL) extracted from a tissue specimen or purified *M. leprae* DNA (positive control) or sterile TE buffer (negative control) was added to the cocktail of amplification to a total amount of 50 μL containing 0.5 μM of each primer, 0.2 mmol/L of each dNTP (dATP, dCTP, dGTP and dTTP) (Bio-Rad), 1 IU of Taq DNA polymerase (Qiagen), 5 μL of 10X PCR buffer (200 mmol/L Tris-HCl pH 8.4, 500 mmol/L KCl), and 1.5 mmol/L $MgCl_2$ (Bio-Rad). Our unpublished experiment found that a $MgCl_2$ concentration of 1.5 mmol/L gave the best results in this PCR protocol. The PCR was performed in a Bio-Rad automated Thermal Cycler (iCycler model) with an initial denaturation step at 95°C for 3 min followed by 40 cycles (denaturation at 95°C for 20 s, annealing at 55°C for 20 s and elongation at 72°C for 30 s) and a final extension at 72°C for 5 min. The amplified product was detected by electrophoresis on a 1.5% agarose gel (Bio-Rad). The DNA was stained with ethidium bromide and visualized on a 302-nm ultraviolet transiluminator (Bio-Rad). The PCR results were determined by the presence or absence of the specific DNA band. The results were further semiquantified as weak positive (1+) or strong positive (2+) according to the brightness of the amplified DNA band by comparison with the control positive band amplified from 0.1 μg purified *M. leprae* DNA.

RESULTS

Sensitivity and specificity of PCR

We first evaluated the specificity of the PCR method using two different primer sets. The amplifications using primers P1–P3 were universal to all mycobacterial species, whereas amplification using P2–P3 was specific to *M. leprae.* To test the specificity, we

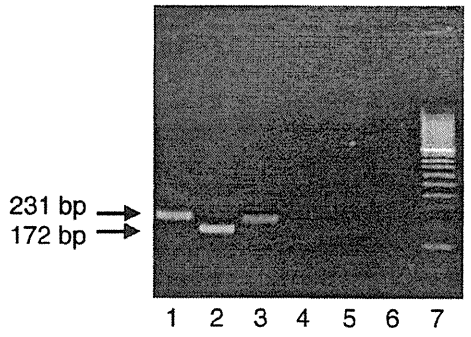

Figure 1. Specificity of polymerase chain reaction (PCR) primers to detect *Mycobacterium leprae*. Purified DNA from *M. leprae, Mycobacterium smegmatis* and *Escherichia coli* were subjected to PCR amplification using primer pairs P1–P3 or P2–P3 as described in Methods. Samples were analyzed by 1.5% agarose gel electrophoresis and specific 231-bp (primers P1–P3) and 172-bp (primers P2–P3) DNA was visualized under ultraviolet light by ethidium bromide staining. Lanes 1 and 2 for *M. leprae* DNA, lanes 3 and 4 for *M. smegmatis* DNA, lanes 5 and 6 for *E. coli* DNA, and lane 7 for 100-bp DNA ladder. Primers P1–P3 were used for lanes 1, 3 and 5, and primers P2–P3 for lanes 2, 4 and 6.

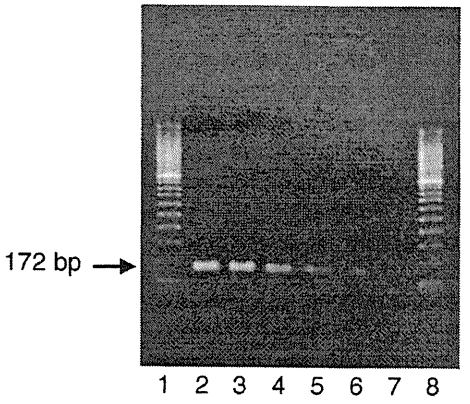

Figure 2. Determination of end-point detection limit of *Mycobacterium leprae* DNA by polymerase chain reaction (PCR). Purified *M. leprae* DNA was serially diluted and subjected to PCR amplification using primers P2–P3 as described in Methods. Samples were analyzed by 1.5% agarose gel electrophoresis and specific 172-bp DNA was visualized under ultraviolet light by ethidium bromide staining. Lanes 2–7: *M. leprae* DNA equivalent to 300 pg, 30 pg, 3 pg, 300 fg, 30 fg and 3 fg, respectively. Lanes 1 and 8: the 100-bp DNA ladder.

utilized DNA from *M. leprae*, *M. smegmatis* and *E. coli* as PCR templates. As expected, primers P1–P3 detected DNA from *M. leprae* and *M. smegmatis* as a specific 231-bp band (Fig. 1, lanes 1 and 3, respectively). Also as expected, primers P2–P3 detected only *M. leprae* as a specific 172-bp band, but not *M. smegmatis* (Fig. 1, lanes 2 and 4, respectively). Neither primer pair amplified *E. coli* DNA (Fig. 1, lanes 5 and 6). These results indicate that the PCR protocol and primers employed in the present study provide reliable evidence to detect *M. leprae* DNA.

We next evaluated the detection limitation by the PCR protocol employed in this study using serial 10-fold dilution of purified *M. leprae* DNA as templates. *M. leprae* genomic DNA (300 pg to 3 fg) were amplified under the conditions described in Methods using P2–P3 primers. As shown in Figure 2, the amount of final PCR product corresponded well with the amount of template *M. leprae* DNA used. The result suggests that it is possible to estimate the amount of template DNA based on the brightness of the specific band on agarose gel in the conditions used in this study. The amplified PCR products were detectable at 30 fg (Fig. 2, lane 6), but not at 3 fg (Fig. 2, lane 7) of *M. leprae* DNA. Given that the size of the *M. leprae* genome is 3.27 Mb corresponding to a weight of 3.6 fg,[19] we estimate that our PCR protocol was able to detect *M. leprae* DNA from at least 8.3 bacilli.

Comparison of PCR with other conventional methods for the diagnosis of leprosy

Typical results of PCR on clinical samples, which supposedly had different amounts of *M. leprae* as indicated by BI values determined from Fite–Faraco staining, are shown in Figure 3. The sample having the highest BI gave the strongest PCR amplification (Figure 3, lanes 2–13), and the PCR results correlated well with BI evaluated by microscopic examination. Of note, PCR was also positive in half of the samples even though their BI were evaluated as zero; that is, acid fast bacilli had not been detected by Fite–Faraco staining of skin smear samples. The overall results of semiquantitative evaluation of PCR for all the samples with their clinical classification and BI values based on microscopic examination of slit-skin smear samples are summarized in Table 1. All the MB samples were positive for PCR detection of

Table 1. Semiquantitative polymerase chain reaction (PCR) detection of the *Mycobacterium leprae* DNA using biopsy samples from leprosy patients and control group

Patient classification	BI	No. of cases evaluated by semiquantitative PCR			Total
		Negative (percent†)	1+ positive (percent†)	2+ positive (percent†)	
Control (plaque psoriasis)	BI = 0	30 (100)	0 (0)	0 (0)	30
Paucibacillary (PB)	BI = 0	16 (50)	16 (50)	0 (0)	32
	0 < BI ≤ 3+	0 (0)	4 (80)	1 (20)	5
	3+ < BI ≤ 4+	0 (0)	13 (65)	7 (35)	20
Multibacillary (MB)	4+ < BI ≤ 5+	0 (0)	3 (50)	3 (50)	6
	5+ < BI ≤ 6+	0 (0)	3 (50)	3 (50)	6
	Total MB	0 (0)	23 (62)	14 (38)	37

†Value is a percentage of the number of patients in each row.

Table 2. Semiquantitative polymerase chain reaction (PCR) and histopathological detection of acid-fast bacilli in leprosy patients

Histopathological detection of acid fast bacilli	No. of cases evaluated by semi-quantitative PCR			Total
	Negative (percent†)	1+ positive (percent†)	2+ positive (percent†)	
Negative	16 (55)	13 (45)	0	29
Positive	0	26 (65)	14 (35)	40
Total	16	39	14	69

†Value is a percentage of the number of patients in each row.

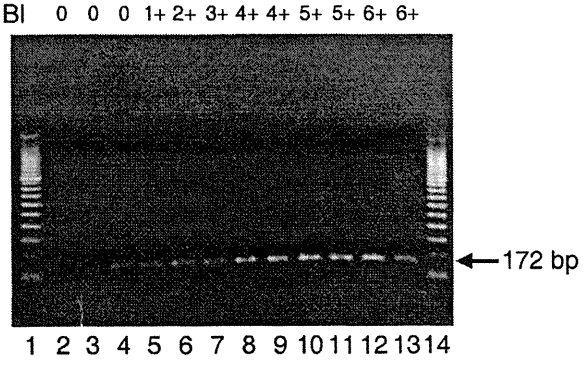

Figure 3. Representative polymerase chain reaction (PCR) results using patient samples. DNA was purified from frozen biopsy specimens and PCR was performed as described in Methods. Samples were analyzed by 1.5% agarose gel electrophoresis and specific 172-bp DNA was visualized under ultraviolet light by ethidium bromide staining. Lanes 2–4 are samples from bacteriological index (BI) = 0 and lanes 6–13 are from samples with various BI. Lanes 1 and 14 are the 100-bp DNA ladder.

M. leprae (37 out of 37 cases). The positive rate for PB cases was 50% (16 out of 32 cases). There was no positive case in plaque psoriasis patients selected as a control group; that is, the specificity was 100%. These results suggest that PCR-based detection of *M. leprae* employed in this study will be sensitive to detect leprosy patients even if the sample is negative for acid-fast staining. Samples with a BI of zero were either negative or weakly positive for PCR. On the other hand, there were no PCR negative cases in the samples with higher BI values.

Histopathological detection of *M. leprae* usually provides superior sensitivity over slit-skin smear detection of *M. leprae*. Thus, in addition to all smear-positive samples, three smear-negative samples exhibited positive results in histopathological detection in the present study (data not shown). Therefore, we compared PCR results with histopathological evaluation of *M. leprae*. PCR was positive in all histopathologically positive samples and 13 (45%) of 29 histopathologically negative samples (Table 2). Compared to PCR, the sensitivity and specificity of histopathological examination were 75.5% and 100%, respectively.

The MI represents the percentage of solid bacilli and is considered to represent the denaturation or the viability of bacilli. In order to explore the possibility that PCR results might represent such features of *M. leprae*, we attempted to compare the

Table 3. Semiquantitative polymerase chain reaction (PCR) of leprosy patients grouped by morphological index (MI)†

MI groups	PCR semiquantitative results		Total
	No. of 1+ positive cases (percent‡)	No. of 2+ positive cases (percent‡)	
MI = 0%	11 (55)	9 (45)	20
0 < MI ≤ 10%	9 (90)	1 (10)	10
10 < MI ≤ 20%	3 (60)	2 (40)	5
MI > 20%	0 (0)	2 (100)	2
Total	23	14	**37**

†MI was evaluated only for positive MB cases (n = 37). ‡Value is a percentage of the number of patients in each row.

PCR results with MI. However, there was no clear relationship between PCR semiquantitative results and MI (Table 3). We additionally compared the semiquantitative results from PCR and serological examination using an anti-PGL-1 antibody in patient sera (Table 4). Although humoral immune responses are considered to be high in MB patients with a supposedly high level of *M. leprae*, there was no clear correlation between semiquantitative PCR results and serum antibody titer where correspondence was 63.8% (κ = 0.208).

DISCUSSION

Leprosy is defined as a person having one or more of the following features: (i) hypopigmented or reddish skin lesion(s) with definite loss of sensation; (ii) involvement of the peripheral nerves, as demonstrated by definite thickening with loss of sensation; and (iii) skin smear-positive for acid-fast bacilli.[20] Despite the gradually decreasing prevalence and incidence of leprosy, the incidence rate of untypical cases is increasing, calling for more sensitive and specific methods of *M. leprae* detection in referral medical settings. Most leprosy patients are diagnosed based on the results from conventional methods such as clinical examination, skin smear and histopathology. In developing countries, where most new cases are detected,[21] clinical signs and skin smears are still the main tools for the detection of leprosy patients. The WHO has urged the development of simple diagnostic technologies, because that would help facilitate access to a greater number of leprosy patients. In addition to the conventional methods described above, serological examination and nucleic acid probes have been suggested for leprosy diagnosis. Unfortunately, none of these tests have shown sufficient sensitivity or specificity to serve as a routine diagnostic tool for leprosy. The major advantages of PCR over other conventional diagnostic methods are that it is a rapid, specific and sensitive approach for the identification of pathogenic microorganisms. It is possible to utilize crude biological samples for PCR with no need for isolation or growth in culture. This is particularly important when trying to identify organisms that are difficult to be cultured, as exemplified by *M. leprae*.

In our study, primers P2–P3 successfully detected *M. leprae* DNA from 8.3 bacilli, showing a greater sensitivity than described.[18,22] All MB patients had a PCR-positive result, as expected, and more importantly, 50% of PB patients showed detectable *M. leprae* DNA by our PCR amplification. In clinical practice, it is extremely important to detect *M. leprae* on BI-negative samples. Histopathology is still not sufficient as an alternative method to detect patients whose BI is zero, although it is more sensitive than skin smears. The specificity of the detection of

Table 4. Semiquantitative polymerase chain reaction (PCR) and anti-phenolic glycolipid-1 (PGL-1) antibody titer in leprosy patients

Anti-PGL-1 antibody measured by *M. leprae* particle agglutination		No. of cases evaluated by semi-quantitative PCR			
		Negative	1+ positive	2+ positive	Total
Negative		10	11	8	29
Positive	×32	3	17	2	22
	×64	1	6	2	9
	>×128	2	5	2	9
	Total positive	6	28	6	40

M. leprae DNA by the present PCR protocol was 100%, probably because the P2 primer is located on a unique nucleotide sequence of *M. leprae.*[16] Another advantage of these primers is that they amplify a short product, which is more likely to result in successful PCR amplification of damaged bacilli that possibly have fragmented DNA.

We have attempted to explore the relationship between semiquantitative PCR and BI or MI. As expected, the positive levels of PCR showed a direct correlation with BI. This could be explained by the fact that BI represents the mycobacterial load in the skin lesion, so that a higher BI indicates more *M. leprae* DNA to be extracted from the lesion, which in turn produce a larger amount of PCR products. On the contrary, there was no relationship between PCR results and MI. This result might reflect the notion that MI represents mycobacterial degradation (either naturally or due to MDT regimen) that occurs in the bacterial cytoplasm, whereas some of the nucleic acid fragments still remain intact within the peptidoglycan layer.[23] Our study did not show a correlation between PCR and serological analysis of anti-PGL-1 antibody using MLPA. This might reflect the humoral immune status of the patient.

There are two molecular methods that can be used for monitoring patients following treatment: reverse transcription (RT)-PCR and longitudinal comparison of signals from DNA-PCR by dimensional densitometry. It was shown in a previous study that *M. leprae* DNA was detectable from blood of patients who had completed MDT for many years by the PCR method.[24] Whether such DNA is derived from circulating fragments of DNA or from viable bacilli still remaining in circulation is not clear at this point.

In summary, our results clearly demonstrate that PCR is a sensitive and specific tool for the diagnosis of leprosy. In BP cases, whose BI is zero, the sensitivity of 50% is one of the advantages of PCR method. However, efforts should be made to improve this sensitivity. On the contrary, the results suggest that PCR should not be used for the purpose of follow up during MDT treatment, because *M. leprae*-derived DNA from severely degenerated bacilli would also be detected by PCR, which does not correlate with MI.

ACKNOWLEDGMENTS

This work was supported in part by an International Cooperation Research Grant, Bureau of International Cooperation, International Medical Center of Japan (IMCJ) to N. I. The authors thank Dr Yasuko Yogi for providing purified *M. leprae* DNA obtained from experimentally infected mouse foot pads, purified *M. smegmatis* DNA and purified *E. coli* DNA.

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Letter to the Editor

No involvement of non-synonymous *TLR2* polymorphisms in Japanese leprosy patients

Leprosy (Hansen's disease) is a chronic infectious disease caused by *Mycobacterium* (*M.*) *leprae*, and primarily affects the skin and the peripheral nerve. Although *M. leprae* are weak in its infectious potency, leprosy has been feared because of the accompanying visible disfigurement and functional defects. In Japan, leprosy patients had been forcedly isolated in leprosaria for 100 years based on the Law for Prevention of Leprosy. Although the Law has been repealed in 1996, most of the patients are already too old to live apart from leprosaria. Nowadays, only few Japanese patients newly occur, whereas 700,000 leprosy patients are still added worldwide per year, mostly in the developing countries.

The clinical and histopathological manifestations of leprosy are varied as a result of immunological responses of a host to *M. leprae*. A number of bacilli are obvious in tissues of the lepromatous (L-type) leprosy patients due to defective cellular immunity, whereas excessive cellular immunity causes epithelioid cell granuloma containing few bacilli in lesions of the tuberculoid (T-type) leprosy patients. Between two poles of the LL- and the TT-types, sequential borderline types (BL/BB/BT) are distinguished according to the Ridley–Jopling classification. Since T helper (Th) 1-type cytokines in the T-type lesions and those of Th2-type in the L-type are dominant, leprosy has been investigated as a good human model of Th1/Th2 diseases. Both genetic and environmental factors are assumed to have roles at two steps of leprosy progression, disease susceptibility and phenotypic difference.

Recently, toll-like receptors (TLRs) have been shown to play a critical role in immunity. They recognize particular molecular patterns of diverse microorganisms to induce strong innate immune responses and provide a critical step for activation of effective adaptive immunity. Among at least 12 TLRs identified in the mammals, TLR2 mediates the immune responses against mycobacteria [1]. Especially, in responses against *M. leprae*, the TLR1/2 heterodimer has a major role [2].

Based on these backgrounds, contribution of the *TLR2* variations to the susceptibility for leprosy has been investigated. In 2001, association of a C-to-T transition at nucleotide position 2029 (*2029C > T*) of *TLR2*, causing a change of arginine at amino acid position 677 to tryptophan (R677W), with the LL-type leprosy was reported in the Korean population [3]. The authors further reported that *M. leprae*-stimulated blood mononuclear cells of the LL-type leprosy patients harboring this mutation produced lower levels of IL-2, IL-12, IFN-γ and TNF-α and a higher level of IL-10, compared with those of the patients without the mutation [4]. Moreover, using the TLR2-transfected cultured cells, impaired NF-κB activation induced by *M. leprae* through this mutant TLR2 was reported by another group, indicating that this mutation is actually functional [5]. Additionally, this *TLR2* polymorphism is reportedly associated with susceptibility to tuberculosis in the Tunisian population [6]. In contrast, an Indian group showed that the primer pairs used in the first Korean report amplified both of the *TLR2* gene and a pseudogene located in its upstream, and detected no such mutation in any Indian leprosy patient using another primer specifically annealing to the *TLR2* gene [7]. Although they suggested that the *2029C > T* mutation is not a true one, the possibility still remains that the different results of these reports reflect the different genetic backgrounds among races.

Therefore in this study, we have investigated the *TLR2 2029C > T* mutation in the Japanese leprosy patients, whose genetic background is close to that of the Korean patients, using both primer pairs used in the Korean report and the Indian one. Furthermore, additional seven known non-synonymous single nucleotide polymorphisms (SNPs) of *TLR2* have been analyzed, including *1232C > T* (T411I), *1667T > C* (I556T), *1736G > A* (R579H), *1892C > A* (P631H), *2143T > A* (Y715N), *2145T > G* (Y715stop), *2258G > A* (R753Q). Notably, the last one is reportedly associated with tuberculosis [8]. The study was performed according to the declaration of Helsinki and approved by the local ethical committee of each institution. Written informed consent for the genetic analysis was obtained from 99 unrelated leprosy patients in National Sanatoria Nagashima Aisei-en and Tama Zensho-en. Except for the BB-type and those of the Korean origin, samples of 60 L-type (46 men and 14 women, 76 as the mean years of age) and 33 T-type patients (22 men and 11 women, 78 as the mean years of age) were analyzed. Genomic DNA was extracted from whole blood of the patients using Genomic DNA Purification Kit (Promega, Madison, WI). The *TLR2* fragments were amplified by polymerase chain reaction (PCR) using Ex-Taq (TAKARA, Otsu, Japan) and Applied Biosystems 2720 Thermal Cycler (Applied Biosystems. Foster City, CA). The nucleotide sequence of amplified DNA fragments was determined by direct sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit and ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Primers used for PCR and sequencing are shown in Table 1.

As a result, the nucleotide position 2029 of all leprosy patients showed C/T heterozygosity using the primer pairs used in the Korean report, whereas the same position of all patients showed C homozygosity using the primer pairs designed by the Indian group (Fig. 1). None of other seven SNPs was identified in any leprosy patient.

Our study clearly showed that the *TLR2 2029C > T* mutation was undetectable in the Japanese leprosy patients similar to the case in the Indian patients, indicating that the previous confusing reports were not due to the ethnical difference. Moreover, none of other non-synonymous *TLR2* SNPs has been identified in our Japanese leprosy patients. Considering the critical role of TLR2 against *M. leprae* infection, those who harbor non-synonymous *TLR2* mutations with functional defects might have been dismissed. Rather, as shown in the recent reports, association of polymorphisms in the promoter and introns as well as synonymous SNPs might be hidden [9,10]. Another possibility would be that polymorphisms

Table 1
Primer pairs used for PCR amplification and sequencing.

	Nucleotide sequence	Nucleotide position
Forward-1	5′-CTGGCCCTCTCTACAAACTT-3′	1155–1174
Reverse -1	5′-TGCACCACTCACTCTTCACA-3′	2121–2140
Forward-2	5′-CCTCCCTCTTACCCATGTTACTA-3′	1484–1506
Reverse-2	5′-CAAATGACGGTACATCCACG-3′	2448–2467

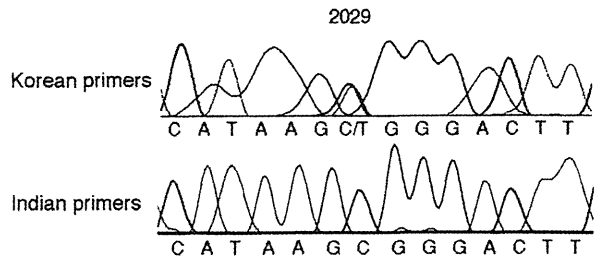

Fig. 1. Sequence analysis of the nucleotide position 2029 of the *TLR2* gene. Genomic DNA of all leprosy patients showed C/T heterozygosity and C homozygosity at the nucleotide position 2029 of the *TLR2*, when analyzed with the primer pairs used in the Korean report (Korean primers) and those used in the Indian report (Indian primers), respectively.

of other class of pattern recognition receptors, such as NOD-LRR proteins and C-type lectins, have stronger association.

In conclusion, no known non-synonymous SNP of *TLR2* has been identified in the Japanese leprosy patients. Further study is necessary to clarify the role of genetic variations of pattern recognition receptors in Japanese leprosy.

Acknowledgements

We would like to thank Ms. Yumi Nakatani and Mrs. Nazuki Nakamura for technical assistance, and Ms. Kazumi Maio for secretary help. This work was supported by the research grant of the Kanae Foundation for the Promotion of Medical Science to N.K.

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9 October2008
4 November 2008

doi:10.1016/j.jdermsci.2008.11.001

Letter to the Editor

Altered expression of fatty acid desaturases in the skin of dogs with atopic dermatitis

Canine atopic dermatitis (AD) is a chronic inflammatory (skin) disease which shares several characteristics with its human counterpart e.g. the genetic predisposition to develop the disease, the early age of onset, the predilection sites of the affected skin and similarities in immunopathogenic mechanisms [1]. Sinke et al. [1] reviewed the immune dysregulation of canine AD and stated that, similar to human AD, it is probably the resultant of a systemic component, the atopic constitution, and a tissue-specific component, i.c. altered reactivity of the skin [2]. With respect to altered skin reactivity both allergen-specific cellular mechanisms and an impaired epidermal barrier in atopic subjects likely contribute to the onset and perpetuation of AD in man [3]. In the beginning of the last century it was proposed that AD is related to abnormal FA metabolism since linoleic acid (LA) deficiency in human and rodents leads to marked abnormalities of the skin of AD patients [4]. More recent studies confirm changes in the lipid organization of the stratum corneum of AD patients [5]. It has been well established that in AD patients LA concentrations tend to be elevated in blood and adipose tissue, however, several studies reported that the levels of downstream metabolites of LA and also of α-linolenic acid (ALA) were found to be reduced [6,7]. Both delta-5-desaturase (FADS1) and delta-6-desaturase (FADS2) are responsible for the synthesis of highly unsaturated *n*-3 and *n*-6 FA from LA and ALA (Fig. 1.). Thus deficit amounts of LA and ALA metabolites in AD have been attributed to reduced Δ-6- and Δ-5 desaturase activity [6]. Human, rat and guinea pig epidermis have been shown to lack enzymatic activity of both desaturases which implies that several important members of epidermal fatty acids, e.g. arachidonic acid (AA), are derived from extra-epidermal sites [8]. To date very few studies focused on the characteristics and metabolism of skin lipids in dogs with respect to a possible epidermal lipid barrier defect in canine AD. We hypothesize that an abnormal lipid metabolism contributes to the pathogenesis of canine AD, potentially as a result of a defect in the epidermal lipid barrier. The aim of the present study was to find evidence for this association in dogs by the analysis of the mRNA expression of these enzymes and the PUFA composition in non-lesional skin (NLS) and lesional skin (LS) of atopic dogs in comparison to healthy controls. Gene expression levels of Δ-5 desaturase (FADS1) and Δ-6 desaturase (FADS2) were measured by quantitative PCR in biopsies from non-lesional and lesional skin of canine AD patients ($n = 28$) and from control skin of healthy dogs ($n = 7$). The mRNA expression level of FADS1 was significantly lower in lesional skin compared to healthy control skin (5.5-fold) and non-lesional atopic skin (4-fold) (Fig. 2 (A)). With respect to FADS2 mRNA expression a significant decrease (1.5-fold) was found in lesional AD skin when compared to non-lesional AD skin (Fig. 2B). Both FADS1 and FADS2 show high correlation in expression in non-lesional ($n = 0.57$; $p < 0.01$) as

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Letter to the Editor

No involvement of the NOD1 polymorphism Glu266Lys in Japanese leprosy patients

Leprosy (Hansen's disease) is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*) and primarily affects the skin and the peripheral nervous system. In Japan, occurrence of new patients is quite rare and most patients are already very old to live in or close to the leprosaria, although more than 200,000 patients newly arise per year in the whole world [1]. Leprosy shows apparent phenotypic heterogeneity depending on the host's immunological response to the pathogen. One is the tuberculoid (T-) type, characterized by formation of epithelioid cell granuloma without apparent bacilli due to the host's strong cell-mediated immunity, and the other is the lepromatous (L-) type, characterized by lipoid degeneration accompanied with multiple bacilli due to the host's defective cell-mediated immunity.

As one cause of the host's response, genetic predisposition has been considered at two stages of the disease progression, including establishment of the disease and determination of the phenotype. Several candidate genes have been investigated with the genome-wide linkage analysis and the case–control study, and some of them have actually been revealed to have an association with leprosy at a distinct stage in a particular ethnic population, including genes of pattern recognition receptors (PRRs), especially toll-like receptors (TLRs) [2]. However, we could not discover any known non-synonymous polymorphism of *TLR2*, the major cell surface PRR for *M. leprae*, in Japanese leprosy patients [3].

On the other hand, sarcoidosis is defined as the systemic granulomatosis with unknown etiology, despite the presence of characteristic epithelioid cell granuloma quite similar to that observed in the T-type leprosy. *Mycobacterium* and *Propionibacterium* species were assumed to have a role in its pathogenesis [4,5] and it has been reported recently, that a genetic polymorphism of *NOD1*, one of the cytosolic receptors for bacterial components, correlated with the incidence of sarcoidosis in the Japanese population [6].

Nod1 and Nod2 are cytoplasmic PRRs recognizing components of cell wall peptidoglycan (PGN) and members of the emerging Nod-like receptor (NLR) family. Nod2 recognizes muramyl dipeptide (MDP) as the minimum component of Lys-type PGN, which is commonly contained in both gram-negative and gram-positive bacteria, and mutations of this gene are reportedly associated with three different granulomatous diseases, Crohn's disease, Blau syndrome and early-onset sarcoidosis [7]. In contrast, Nod1 had been considered to specifically recognize γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP) as the minimum component of DAP-type PGN exclusively contained in gram-negative bacteria. However, smaller components, *meso*-DAP in mycobacteria and LL-DAP in *Propionibacterium acnes* (*P. acnes*), have been shown to activate Nod1 [8]. Furthermore, the Nod1 with a substitution of glutamic acid at amino acid position 266 to lysine (E266K) caused by the nucleotide change at position 796 from guanine into adenine (796G>A), which is reportedly associated with sarcoidosis in Japan, has been revealed to impair the NF-κB activation induced by intracellular *P. acnes* [6].

Therefore, in this study, possible association of the *NOD1 796G>A* with leprosy in the Japanese population was investigated. No other non-synonymous polymorphism of *NOD1* than *796G>A* had been reported in the database of Japanese Single Nucleotide Polymorphisms. Genomic DNA extracted from whole blood of unrelated leprosy patients in National Sanatoria Nagashimaaiseien and Tamazenshoen and healthy volunteers of the Japanese origin was analyzed after obtaining the agreement for the genetic analyses. Informed consent was provided according to the Declaration of Helsinki and the study was approved by the Ethics Committees of Wakayama Medical University and Tamazenshoen. 60 L-type and 33 T-type patients and 50 controls were included in the study, containing 46 men and 14 women (76 years as the mean age) and 22 men and 11 women (78 years) and 27 men and 23 women (42 years), respectively. The *NOD1* fragment containing nucleotide position 796 was amplified and directly sequenced using the following primers; 5′-TCAGGAATGCTGGCTCTGTG-3′ and 5′-AGCCCAGCTGCATGTCTCTC-3′ for polymerase chain reaction and 5′-TGAGCAGGGTGAGACCATC-3′ for sequencing.

The resulting genotype frequencies of the *NOD1* nucleotide position 796 polymorphism (GG/GA/AA) among patients of each leprosy type and healthy control are summarized in Table 1. By the chi-square test, no significant difference of the appearance of each genotype was observed between the L-type and the T-type ($p = 0.996$), as well as between all leprosy patients and the healthy individuals ($p = 0.4346$) in the presence of considerable difference between their mean ages. No significant difference was detected in either of allele frequency or appearance of genotype in any inheritance model (data not shown).

Thus, our study revealed no association of *NOD1 796G>A* polymorphism with the Japanese leprosy patients. As this report is the first that investigated the association of *NOD1 796G>A* with leprosy and the number of our study subjects was only limited, further study would be necessary to make a conclusion. However, considering the same ethnic background of our study with the previous study on sarcoidosis [6], role of Nod1 in leprosy seems different from that in sarcoidosis in spite of the possible pathophysiological similarities between these diseases. Since it has not been determined whether Nod1 is actually involved in recognition of *M. leprae*, it might be possible that Nod1 is involved in intracellular recognition of *P. acnes* but *not M. leprae*.

More recently, *NOD1 796G>A* polymorphism has been reported to be associated with the susceptibility to Crohn's disease in the Hungarian population [9]. Considering the possible role of *Mycobacterium* species in Crohn's disease [10], it is probable that *NOD1* polymorphism may have a role in leprosy in other ethnic populations and/or may be involved in intracellular recognition of some other mycobacteria.

0923-1811/$36.00
doi:10.1016/j.jdermsci.2009.07.004

Table 1
Genotype frequencies of *NOD1* polymorphism in leprosy patients and healthy control.

Group	*n*	The number (%) of each genotype			χ^2
		GG	GA	AA	
Leprosy patients					
L-type	60	16 (27)	29 (48)	15 (25)	$p = 0.9960$
T-type	33	9 (27)	16 (49)	8 (24)	
All	93	25 (27)	45 (48)	23 (25)	$p = 0.4346$
Healthy control	50	9 (18)	29 (58)	12 (24)	

L: lepromatous, T: tuberculoid.

In conclusion, no association of NOD1 Glu266Lys polymorphism has been revealed with the Japanese leprosy patients.

Acknowledgements

We would like to thank Mrs. Nazuki Nakamura for technical assistance and Ms. Kazumi Maio for secretary help. This work was supported by the research grant of the Kanae Foundation for the Promotion of Medical Science to N.K.

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10 March 2009

Jpn.J.Leprosy 78, 25-34(2009)

2008年における世界のハンセン病の現況について

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〔受付・掲載決定：2008年10月14日〕

キーワード：世界保健機関、多剤併用療法、ハンセン病新規患者、ハンセン病制圧、有病率

世界のハンセン病の疫学は各国の保健担当の部署から世界保健機関（WHO）に報告される。報告されたデータはWHOによってまとめられ、速報的に週間疫学記録（weekly epidemiological record）に掲載される。2008年8月に、2008年初頭のデータとして報告された。世界のハンセン病制圧は着実に進行しているが、今後患者数が減少した後にも政治的関心を維持し、この分野での活動を実行するために必要な資金を結集することが重要であることなどが述べられている。

世界保健機関（WHO）発行の週刊疫学記録（weekly epidemiological record）、2008年8月15日号（No. 33, 2008, 83, 293-300）（http://www.who.int/wer）に掲載された「世界のハンセン病状況、2008年初頭」（Global leprosy situation, beginning of 2008）についてWHOの許可を受け、日本語訳を行った。

世界のハンセン病の現況をWHO事務局別、各国別で表示してある。各国の報告は国情により内容の信頼性に温度差があるものの、概略はこの報告で伺うことができる。この報告を参考にして、ハンセン病の世界の現況を把握するとともに、我々日本人として行うことができる国際協力に関して考察を頂ければ幸いである。また表については原文のまま記載した。

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世界のハンセン病現況：2008年初頭

2008年の初頭現在、多くの流行国でのハンセン病対策プログラムはそれらの活動を維持し続けるのに成功している。これらの努力の成果により、2007年の世界の新規患者数は減少し続けている。

*ハンセン病問題のさらなる減少と制圧活動維持のための世界戦略案（計画期間2006－2010）*とその実行のためのガイドラインは全てのWHO地域において広く実行されている。ハンセン病が流行している多くの国々において、ハンセン病問題を減少させるのに効果的と証明されてきた、新規患者の早期発見と多剤併用療法（MDT）による迅速な対応が、これからも主な介入戦略となる。これに加えて、制圧活動と一般保健サービスの統合は、サービス範囲とケアの質の改善の両面で国家プログラムの維持を可能にした。

制圧プログラムが達成された後でも政治的関心を維持し、この分野での活動を実行するために、

必要な資金を集めることは、ハンセン病問題が改善された時においても多くの国家プログラム中で優位に立てる。

ハンセン病問題

2008年の初頭、世界におけるハンセン病登録患者数は212,802人、2007年の新規患者数は254,525人であった（Table 1）。2007年の世界における新規患者数は2006年に比べると11,100人（4%）以上減少した。

Table 2は2001-2007年の期間の地域ごとの新規患者数を示している。世界的にみると、年間新規患者数は最高時の2001年の763,000人から2007年の254,525人と減少し続けている。新規患者数のデータはマラウィとタンザニアを含む流行国から現在も収集中である（訳注：2007年の新規患者は増加すると考えられる）。2007年の東地中海地域における新規患者数は、ハンセン病制圧活動の範囲が大いに向上した南スーダンからの報告もあったため増加している。

2008年初頭にコンゴ民主共和国とモザンビークはハンセン病制圧目標を達成した（登録患者数が人口10,000人あたり1人未満と定義される）。Table 3は人口が100万人以上の国の中で未だに制圧目標を達成していない3ヶ国のハンセン病の有病率と新規患者数を示す（ブラジル、ネパール、東ティモール）。

合わせて、これらの3ヶ国は2007年の新規患者数の約17%、そして2008年初頭の登録患者数の23%を占める。GOやNGO（パートナー）からの援助や国家機関の協力の持続によって、これらの3ヶ国は近い将来に制圧目標を達成するだろう。

2007年において1,000人以上の新規患者数のいる国は17ヶ国であった(Table 4)。これらの国々は2007年における世界の総新規患者数の95%を

Table 1. Prevalence of leprosy and number of new cases detected, by WHO region, beginning of 2008

WHO region[a]	Registered prevalence,[b] beginning 2008	New cases detected,[c] 2007
African	30 055 (0.47)	31 037 (4.85)
Americas	49 388 (0.96)	41 978 (8.15)
South-East Asia	120 967 (0.72)	171 552 (10.22)
Eastern Mediterranean	4 240 (0.09)	4 091 (0.85)
Western Pacific	8 152 (0.05)	5 867 (0.34)
Total	212 802	254 525

[a] Population data from *World population prospects: the 2004 revision*, Vol. 1, Table A20. New York, United Nations Population Division. Data from the European Region are not included.
[b] Values are number (rate/10 000 population).
[c] Values are number (rate/100 000 population).

Table 2. Trends in the detection of new cases of leprosy, by WHO region, 2001–2007 (excluding European Region)

WHO region	No. of new cases detected						
	2001	2002	2003	2004	2005	2006	2007
African	39 612	48 248	47 006	46 918	45 179	34 480	31 037
Americas	42 830	39 939	52 435	52 662	41 952	47 612	41 978
South-East Asia	668 658	520 632	405 147	298 603	201 635	174 118	171 552
Eastern Mediterranean	4 758	4 665	3 940	3 392	3 133	3 261	4 091
Western Pacific	7 404	7 154	6 190	6 216	7 137	6 190	5 867
Total	763 262	620 638	514 718	407 791	299 036	265 661	254 525

占める。

新規患者が100人以上の国を対象としてWHO地域ごとの新規患者の詳細をTable 5に示す。全ての地域の国々において、新規患者の中の多菌型ハンセン病患者数（multibacillary: MB）、子供、女性、第2級障害者（grade 2 disabilities:G2D）の割合にはばらつきが大きく存在する。

アフリカ地域では、MB患者の割合はコモロの40.74%からエチオピアの92.93%まで及ぶ。アメリカ地域では、この割合はブラジルの53.53%からパラグアイの78.66%に及ぶ。南東アジア地域では、バングラデシュは43.81%から、インドネシアの79.6%に及ぶ。東地中海地域では、ソマリアの29.71%からエジプトの88.73%まで及ぶ。西

Table 3. Prevalence of leprosy and number of new cases detected in countries with population >1 million that have not yet eliminated the disease

Country	Registered prevalence[a]			No. of new cases detected[b]		
	Beginning of 2006	Beginning of 2007	Beginning of 2008	2005	2006	2007
Brazil	27 313 (1.5)	60 567 (3.21)	45 847 (2.40)	38 410 (20.6)	44 436 (23.53)	39 125 (20.45)
Nepalc	4 921 (1.8)	3 951 (1.43)	3 329 (1.18)	6 150 (22.7)	4 253 (15.37)	4 436 (15.72)
Timor	289 (3.05)	222 (2.2)	131 (1.23)	288 (30.41)	248 (24.63)	184 (17.23)

[a] Values are number (rate/10 000 population).

[b] Values are number (rate/100 000 population).

[c] Detection reported for mid-November 2006 to mid-November 2007.

Table 4. Detection of new cases of leprosy in 17 countries reporting ≥1 000 new cases during 2007 and the number of new cases detected previously

Country	No. of new cases detected						
	1993	2002	2003	2004	2005	2006	2007
Angola	339	4 272	2 933	2 109	1 877	1 078	1 269
Bangladesh	6 943	9 844	8 712	8 242	7 882	6 280	5 357
Brazil	34 235	38 365	49 206	49 384	38 410	44 436	39 125
China	3 755	1 646	1 404	1 499	1 658	1 506	1 526
Democratic Republic of he Congo	3 927	5 037	7 165	11 781	10 369	8 257	8 820
Côte d'Ivoire	2 186	1 358	1 205	1 066	NA	976	1 204
India	456 000	473 658	367 143	260 063	169 709	139 252	137 685
Ethiopia	4 090	4 632	5 193	4 787	4 698	4 092	4 187
Indonesia	12 638	12 377	14 641	16 549	19 695	17 682	17 723
Madagascar	740	5 482	5 104	3 710	2 709	1 536	1 644
Mozambique	1 930	5 830	5 907	4 266	5 371	3 637	2 510
Myanmar	12 018	7 386	3 808	3 748	3 571	3 721	3 637
Nepal	6 152	13 830	8 046	6 958	6 150	4 235	4 436
Nigeria	4 381	5 078	4 799	5 276	5 024	3 544	4 665
Philippines	3 442	2 479	2 397	2 254	3 130	2 517	2 514
Sri Lanka	944	2 214	1 925	1 995	1 924	1 993	2 024
Sudan	1 489	1 361	906	722	720	884	1 706a
Total (%)	555 209 (94%)	594 849 (96%)	490 494 (95%)	384 409 (94%)	282 897 (95%)	245 626 (95%)	240 032 (94%)
Global total	590 933	620 638	514 718	407 791	299 036	259 017	254 525

NA, not available.

[a] Total for 2007 includes data from southern Sudan.

太平洋地域では、ミクロネシア連邦の46.1%から中国の84.93%に及ぶ。

アフリカ地域の新規患者数の女性の割合はマダガスカルの25.58%からコンゴ共和国の60.15%まで及ぶ。アメリカ地域では、エクアドルの18.69%からブラジルの44.84%まで及ぶ。南東アジア地域では、ネパールの30.68%から東ティモールの63.59%まで及ぶ。東地中海地域ではソマリアの28.74%からスーダンの46.25%も及ぶ。西太平洋地域では、フィリピンの20.01%からラオスの77.6%までである。

新規患者の中の子供の割合も広いばらつきを見せる。アフリカ地域ではトーゴの2.89%からコモロの37.96%まで及ぶ。アメリカ地域ではアルゼンチンの0.32%からドミニカ共和国の14.02%まで及ぶ。南東アジア地域では、ネパールの3.34%から東ティモールの14.1%まで及ぶ。東地中海地域ではスーダンの3.63%からイエメンの13.6%まで及ぶ。西太平洋地域では、中国の2.23%からミクロネシア連邦の26.95%までである。

また同様に、新規患者の中のG2Dの割合も幅広いばらつきを見せる。アフリカ地域では、ガーナの1.85%からブルンジの25.52%に及ぶ。アメリカ地域では、キューバの3.28%からコロンビアの9.8%まで広がる。南東アジア地域ではネパールの2.14%からミャンマーの13.2%に及ぶ。東地中海地域ではソマリアの5.8%からパキスタンの19.4%に及ぶ。西太平洋地域ではミクロネシア連邦の0%から中国の22.7%に及ぶ。

Table 6は、2008年初頭の登録患者数、2007年に発見された新規患者数、新規MB患者数、新規患者の女性や小児患者の数、新規患者のG2D患者数、再発患者の絶対数、そして少菌型（PB）とMBの治癒率をについて118の国や地域からのデータを示す。報告はアフリカ地域の32の国や地域から、アメリカ地域では30、南東アジア地域では8、東地中海地域では19、そして西太平洋地域で29ヵ所から集められた。基礎となる人口は国連人口部門の2006年人口データに基づいており、それらを用いて比率は計算された。

2007年に報告した118の国や地域のうち、65カ国は年間0人から100人未満の新規患者数であった。

Table 5. Profile of newly detected cases of leprosy reported by countries with ≥100 new cases, by WHO region, 2007

WHO region	% multibacillary among new leprosy cases by countries with highest and lowest proportions	% of females among new leprosy cases by countries with highest and lowest proportions	% of children among new leprosy cases by countries with highest and lowest proportions	% of new leprosy cases with grade 2 disabilities by countries with highest and lowest proportions
African	Comoros, 40.74% Ethiopia, 92.93%	Madagascar, 25.58% Congo, 60.15%	Togo, 2.89% Comoros, 37.96	Ghana, 1.85% Burundi, 25.52%
Americas	Brazil, 53.53% Paraguay, 78.66%	Ecuador, 18.69% Brazil, 44.84%	Argentina, 0.32% Dominican Republic, 14.02% –	Cuba, 3.28% Colombia, 9.8%
South-East Asia	Bangladesh, 43.81% Indonesia, 79.60%	Nepal, 30.68% Timor-Leste, 63.59%	Nepal, 3.34% Timor-Leste, 14.1%	Nepal, 2.14% Myanmar, 13.2%
Eastern Mediterranean	Somalia, 29.71% Egypt, 88.73%	Somalia, 28.74% Sudan, 46.25%	Sudan, 3.63% Yemen, 13.6%	Somalia, 5.8% Pakistan, 19.4%
Western Pacific	Micronesia (Federated States of), 46.1% China, 84.93%	Philippines, 20.01% Lao People's Democratic Republic, 77.6%	China, 2.23% Micronesia (Federated States of), 26.95%	Micronesia (Federated States of), 0% China, 22.7%

Table 6. Global leprosy situation by WHO region (excluding the European Region) and country or territory, beginning 2008. (Blank cells indicate that no data were available.)

Region and country or territory	Registered prevalence[a]	No. of new cases detected, 2007	No. of new cases of multibacillary leprosy	No. of new female cases	No. of new cases among children	No. of new cases with grade 2 disabilities	No. of relapses 2007	Cure rate (%) PB[b]	Cure rate (%) MB[c]
African									
Algeria	0	0	0	0	0	0	0		
Angola	1 218	1 269	959	663	130	135	0		
Benin	284	345	212	168	41	80			
Botswana	0	0	0	0	0	0	0		
Burkina Faso	578	588	432		30	71			
Burundi	404	239	217	97	11	61	19		
Cameroon	520	549	383	241	18	32	0		
Cape Verde	12	8	7	3	0	1	0		
Central African Republic	366	345	218	163	52	47	4		
Chad	976	631	489	183	26	101	0		
Comoros	70	108	44	48	41	8	4		
Congo	246	261	191	157	30	21			
Côte d'Ivoire	1 165	1 204	817	407	121	152	0		
Democratic Republic of the Congo	6 502	8 820	4 770		1 107	743		92.3	84.85
Ethiopia	4 611	4 187	3 891	1 343	312	411	227		
Gabon	33	27	26	0	0	4	0		
Ghana	621	594	500	269	41	11	0		
Guinea	684	803	551		79	88			
Guinea-Bissau	69	58	40	24	3	10	0		
Lesotho	4	4	4	1	0	0	0		
Liberia	667	319	215	147	44	15	0		
Madagascar	1 591	1 521	1 137	389	212	187			
Mali	439	455	331				0		
Mozambique	1 830	2 510	1 723		221	263	18		
Niger	539	610	443	291	19	72	0		
Nigeria	5 381	4 665	4 188	2 002	417	540	36		
Senegal	433	282	219	117	37	32	17		
Seychelles	0	1	1	1	1	0	0		
South Africa	171	66	63	36	9	18	3		
Togo	158	173	131	80	5	25			
Uganda	472	389	311	189	29	68	8	90.00	80.2
Zimbabwe	11	6	5	2	0	4	0		
Total	30 055	31 037	22 518	7 021	3 036	3 200	336		
Americas									
Anguilla	0	0	0	0	0	0	0		
Antigua and Barbuda	0	0	0	0	0	0	0		
Argentina		312	234	130	1	24	11		
Brazil	45 847	39 125	20 945	17 545	2 966	3 239	1 534		
Bahamas	0	0	0	0	0	0	0		
Barbados	0	0	0	0	0	0	0		
Belize	0	0	0	0	0	0	0		
Bermuda	0	0	0	0	0	0	0		
Cayman Islands	0	0	0	0	0	0	0		
Colombia		510	356		9	50	17		
Costa Rica	45	11	11	6	0	0	23		
Cuba		244	176	109	2	8	6		

Dominica	0	0	0	0	0	0	0		
Dominican Republic	327	164	105	73	23	10	4		
Ecuador	195	107	75	20	1	7	6		
El Salvador	10	8	8	2	0	2	0		
Guyana	56	26	19		7	1		100	100
Haiti		29	16	13	9	0	0		
Jamaica	18	6	2	1	3	0	0		
Mexico	702	243	179	90	2	14	11		
Montserrat	0	0	0	0	0	0	0		
Paraguay	414	403	317	155	17	34	10		
Peru		19	16		1	6			
Saint Lucia	12	12	7	7	0	0	0	100	100
Saint Vincent and the Grenadines	0	0	0	0	0	0	0		
Suriname	36	42	31	21	6	3	0		
Trinidad and Tobago	45	30	16	14	2	1			
Turks and Caicos Islands	0	0	0	0	0	0	0		
Uruguay		4	4	1	0	1	3		
Venezuela (Bolivarian Republic of)	1 681	683	440	236	40	31	23		
Total	**49 388**	**41 978**	**22 957**	**18 423**	**3 089**	**3 431**	**1 648**		
South-East Asia									
Bangladesh	4 463	5 357	2 347	2 077		365	556		
India	87 228	137 685	64 980	47 537	12 976	3 477			
Indonesia	21 430	17 723	14 107		1 824	1 527	91	83.0	84.0
Myanmar	2 892	3 637	2 441	1 256	211	481	4	97.77	95.47
Nepal	3 329	4 436	2 300	1 361	148	95			
Sri Lanka	1 494			2 024	916	907	196	118	8
Thailand		506	322	200	17	58	6	85.44	80.69[d]
Timor-Leste	131	184	119	117	26	20	10		
Total	**120 967**	**171 552**	**87 532**	**53 455**	**15 763**	**6 332**	**119**		
Eastern Mediterranean									
Afghanistan	32	26	22	5	3	4	0		
Bahrain		2	1	0	0	0	0		
Egypt	1 592	887	787	347	53	56	3		
Iran (Islamic Republic of)	182	25	25	13	0	7	12	88.00	89.00
Iraq	0	0	0	0	0	0	0		
Jordan	0	0	0	0	0	0	0		
Kuwait	20	20	14	3					
Libyan Arab Jamahiriya		8	6	2	0	0	0	100.00	100.00
Morocco		38	25	15	2	6	0		
Oman	1	2	0	1	0	1	0		
Pakistan	856	496	377	211	38	96	22		
Saudi Arabia	14	20	13	5	0	1	0		
Somalia	114	414	123	119	17	24	3		
Sudan	940	1 706	1 459	789	62	211	17		
Syria	2	2	2	1	0	0	0		
Tunisia	1	0	0	0	0	0	0		
United Arab Emirates		11		0	0	0			
West Bank and Gaza Strip	0	0	0	0	0	0	0		
Yemen	486	434	262	149	59	60	3	96.12	90.47
Total	**4 240**	**4 091**	**3 116**	**1 660**	**234**	**466**	**60**		
Western Pacific									
American Samoa	5	0	0	0	0	0	1		