Eur Respir J 2007; 30: 90–96
DOI: 10.1183/09031936.00042506

Pulmonary *Mycobacterium avium* complex infection: association with *NRAMP1* polymorphisms

G. Tanaka*, J. Shojima*, I. Matsushita*, H. Nagai#, A. Kurashima¶, K. Nakata*, E. Toyota+, N. Kobayashi+, K. Kudo+ and N. Keicho*

ABSTRACT: The present study aimed to elucidate risk factors for nonimmunocompromised pulmonary *Mycobacterium avium* complex (MAC) infection.

Epidemiological data and variations of candidate genes for mycobacterial diseases were analysed in 111 patients with pulmonary MAC infection. Four polymorphisms of the human natural resistance-associated macrophage protein (*NRAMP*)*1* gene, the 5′(GT)n, 469+14 G/C, D543N and the 3′untranslated region (3'TGTG) insertion/deletion, were genotyped using PCR-based methods. *Fok* I and *Taq* I polymorphisms of the vitamin D receptor gene and -221 X/Y and codon 54 A/B polymorphisms of the mannose binding lectin gene were also evaluated.

Females were more susceptible to MAC infection mainly affecting the right middle lobe or lingular segment of the lung. Patients' residence at the onset of the disease was distributed evenly irrespective of a waterfront or city water supply system. As compared with homozygotes for major alleles of the D543N and TGTG insertion/deletion polymorphism of the *NRAMP1* gene, heterozygotes containing minor alleles were less often observed in MAC cases than in controls. This genetic effect was more significant in patients without comorbidity but not in patients with comorbidity. Other polymorphisms did not show any association with the MAC infection.

The human natural resistance-associated macrophage protein 1 gene might be involved in susceptibility to pulmonary *Mycobacterium avium* complex infection.

KEYWORDS: Mannose binding lectin, *Mycobacterium avium* complex, natural resistance-associated macrophage protein 1, vitamin D receptor

Pulmonary *Mycobacterium avium* complex (MAC) infection causes chronic pulmonary diseases. MAC occurs in the natural environment and the common source of infection appears to be water, soil or dust, with human-to-human transmission considered uncommon [1–3]. As an opportunistic pathogen, MAC causes disseminated disease in immmunocompromised hosts, such as individuals with HIV infection. However, there is evidence that the number of patients with MAC infection is increasing not only in AIDS-endemic areas but also in many other areas of the world, including Japan [4, 5].

Patients with underlying chronic lung diseases, such as inactive tuberculosis (TB), chronic obstructive pulmonary disease or cystic fibrosis, sometimes develop pulmonary MAC infection [5], which may be explained by significant damage to local immunity in the lung. Individuals without any obvious immunosuppressive state or any evidence of previous pulmonary disease, especially middle-aged to elderly females, also develop pulmonary MAC infections [6, 7]. In most patients, a radiographical pattern consisting of small centrilobular nodules corresponding to foci of granulomatous inflammation and bronchiectasis of the middle lobe, lingular segment and other lobes can be seen [8, 9]. These lesions often expand, causing impairment of pulmonary function and, in severe cases where the treatment is difficult, a fatal outcome may occur [6]. A recent study implied that the sibling risk for MAC infection is much higher than its population prevalence estimated from an incidence rate of 3.52 per 100,000 in Japan [4, 10]. A complex interaction between genetic and environmental factors is thus considered.

The natural resistance-associated macrophage protein (*Nramp*)1 gene determines susceptibility to intracellular pathogens in mice [11]. A human homologue, *NRAMP1*, recently designated as solute carrier 11a1, was identified in the region

AFFILIATIONS
*Dept of Respiratory Diseases, Research Institute and
+Division of Respiratory Disease International Medical Center of Japan, and
Divisions of #Respiratory Disease and
¶Clinical Research, National Hospital Organization Tokyo Hospital, Tokyo, Japan.

CORRESPONDENCE
N. Keicho
Dept of Respiratory Diseases
Research Institute
International Medical Center of Japan
1-21-1 Toyama
Shinjuku-ku
Tokyo 162-8655
Japan
Fax: 81 332071038
E-mail: keicho@ri.imcj.go.jp.

Received:
March 25 2006
Accepted after revision:
April 18 2007

SUPPORT STATEMENT
This work was supported in part by research grants for Research on the Human Genome, Tissue Engineering and Food Biotechnology from the Ministry of Health, Labour and Welfare of Japan, by the Organization for Pharmaceutical Safety and Research in 2002 and 2003 and by Grants-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan in 2001–2005.

STATEMENT OF INTEREST
None declared.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

of 2q35, and variations of the *NRAMP1* gene were studied for mycobacterial diseases including TB and leprosy [12–16]. In cases of MAC infection, the number of subjects is small [10, 17, 18], and, to date, no population-based studies have investigated the contribution of *NRAMP1* to pulmonary MAC infection with a relatively large sample size. As other candidate genes, polymorphisms of the vitamin D receptor (*VDR*) and mannose binding lectin (*MBL*) genes are known to be associated with TB [19, 20], presumably playing an important role in intracellular growth of the pathogen. Thus, the clinico-epidemiological background of the disease was characterised and a case–control association study was conducted to determine whether polymorphisms of the three representative candidate genes are involved in the development of pulmonary MAC infection.

MATERIAL AND METHODS

Study subjects

In total, 111 Japanese cases of pulmonary MAC infection were included in the current study. Written informed consent was obtained from each individual. The present study protocol was approved by the local ethical committees. Out of the 111 subjects, 86 patients were from the International Medical Center of Japan and 25 were from the National Hospital Organization Tokyo Hospital (both Tokyo, Japan). A group of 177 healthy Japanese volunteers (control 1) were also obtained from the same region as the patients and were genotyped as controls. Only when a significant association ($p<0.05$) was obtained in control 1, was control 2 ($n=247$) further tested. The 1997 American Thoracic Society (ATS) statement was followed to make a diagnosis of MAC pulmonary disease [3]. Briefly, all patients had clinical manifestations, small nodules with or without bronchiectasis on computed tomography images and positive smears or cultures of bacteria from at least three sputum samples, or histological or bacteriological evidence of the disease from bronchial or lung samples. Patients with obvious immunodeficiency, such as haematological malignancy, those who are under immunosuppressive therapy or those with HIV infection were excluded from this study. Comorbidity was described on the basis of a physician's diagnosis.

Differentiation of cultured mycobaterial species was routinely performed by PCR (AMPLICOR Mycobacterium tests; Roche Diagnostics, Basel, Switzerland). Clinical profiles and backgrounds of all subjects were extracted from medical records and interviews performed by trained medical staff.

Genotyping of NRAMP1 *polymorphisms*

Two polymorphisms of the *NRAMP1* gene, 469+14 G/C in intron 4 (INT4; NCBI dbSNP ID rs3731865) and TGTG insertion and deletion polymorphism in the 3′ untranslated region (3′UTR; rs17235416), were analysed as described previously [21]. For genotyping of a nonsynonymous single nucleotide substitution at codon 543 in exon 15 (D543N; rs17235409), PCR was carried out with previously described primers [21], and the sequencing was performed using the Big Dye Terminator cycle sequencing method using ABI PRISM 3100 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). For the genotyping of GT repeat polymorphisms of the promoter region of *NRAMP1* [12], the PCR primer was designed as follows. The forward primer (5′-ACTCGCATTAGGCCAACGAG-3′) was labelled with fluorescent dye. To avoid ambiguous typing, extra GT nucleotides were added to the 5′ end of the reverse primers (5′-(GT)TTCTGTGCCTCCCAAGTTAGC-3′) [22]. PCR products were genotyped using ABI PRISM 377 DNA Sequencer (Applied Biosystems) according to the manufacturer's instructions. The fluorescent signals were analysed by GeneScan software and genotyped using Genotyper software (Applied Biosystems). Allele names of the GT repeat polymorphisms were designated as described by LIU *et al.* [21].

Genotyping of VDR *and* MBL *polymorphisms*

Two polymorphisms of the *VDR* gene, a C-to-T transition in exon 3 that creates an alternative initiation codon (rs10735810) and a T-to-C substitution in the 3′UTR of exon 10 (rs731236), were genotyped by digestion of PCR fragments with the *Fok* I and *Taq* I enzymes, respectively. PCR primers and enzymatic digestion have been described elsewhere [23].

Two polymorphisms of the *MBL* gene, the -221 X/Y (rs7096206) in the promoter and codon 54 A/B (rs1800450) in exon 1, were genotyped by digestion of PCR fragments with the *Btg I* and *Ban I* enzymes, respectively. The PCR primer pair used was 5′-ACCTGGGTTTCCACTCATTCTCAT-3′ and 5′-CCCCAGGCAGTTTCCTCTGGAAGG-3′. Other known structural polymorphisms, C (codon 57; rs1800451) and D (codon 52; rs5030737), were not tested in the current study, as their frequencies have been reported to be extremely low in Asian populations [24]. All genotypes were determined by agarose gel electrophoresis of digested PCR fragments.

Statistical analysis

Disease association with each polymorphism was analysed by $2\times n$ Fisher's exact test [25]. The current authors also examined whether genotype frequencies were in Hardy-Weinberg equilibrium in control subjects. Haplotype frequencies were estimated with an expectation-maximisation algorithm [25] and haplotype association tested using hapassoc [26]. A p-value <0.05 was considered significant.

RESULTS

Characteristics of patients with pulmonary MAC infection

In total, 111 patients were involved in the current study (table 1). Following the study of PRINCE *et al.* [6], patients with pulmonary MAC infection were initially divided into two groups on the basis of the predisposing disease. The ''MAC with comorbidity'' group consisted of 53 patients with previous lung disease, including TB, chronic obstructive disease, pneumonia or other potential predisposing conditions, such as nonhaematological malignant disease, diabetes mellitus, or post-gastrectomy. The ''MAC without comorbidity'' group consisted of 58 patients who were otherwise normal and had no recognisable predisposing diseases such as those described previously. The average age at onset of disease was 61.9 *versus* 57.4 yrs in patients with and without comorbidity, respectively ($p=0.067$). Females were more susceptible to MAC infection in the group without comorbidity (86.2%) than with comorbidity (67.9%; $p=0.025$). Approximately 90% of patients were infected with *Mycobacterium avium* and the remainder had *M. intracellulare*. The right middle lobe or lingular segment of the lung was mainly affected and this

TABLE 1 Characteristics of patients with pulmonary *Mycobacterium avium* complex (MAC) infection

	Total	MAC with comorbidity	MAC without comorbidity
Cases	111	53	58
Age at onset yrs	59.5±12.5	61.9±12.3	57.4±12.3
Male/female	25/86	17/36	8/50
Smoking history	22	15	7
Mycobacterial species			
M. avium	91	43	48
M. intracellulare	11	5	6
Unknown#	9	5	4
Main lesion of disease			
RML/lingular	45	16	29
RML/lingular and other lobes	38	22	16
Other lobes	28	15	13
History of lung diseases	40	40	
TB	29	29	
COPD	1	1	
Pneumonia	11	11	
Lung cancer	1	1	
Others	5	5	
History of other predisposing diseases	14	14	
Diabetes	7	7	
Neoplasm	5	5	
Gastrectomy	2	2	
Others	2	2	

Data are presented as n or mean±SEM. *M. avium*: *Mycobacterium avium*; *M. intracellulare*: *Mycobacterium intracellulare*; RML: right middle lobe; TB: tuberculosis; COPD: chronic obstructive pulmonary disease. #: MAC with no detailed information.

localisation of the legions was more frequently seen in the MAC patients without comorbidity than with comorbidity, although the statistical significance remained marginal (p=0.052).

Residence at onset of pulmonary MAC infection

To examine whether the development of pulmonary MAC infection is related to an urban water supply, the locations at which the first signs and symptoms of MAC infection were noted, were plotted on a regional map of Tokyo, Japan (fig. 1) [27]. The residential information at the onset of the disease was available from 88 patients. The points where patients resided at the onset of the disease tended to be located in two areas, surrounding each hospital. As expected, there was a similar distribution of patients living in both areas. These areas were either supplied by the Tonegawa river water system or by a mixture of water systems from both the Tonegawa and Tamagawa rivers. The points of residence were not particularly concentrated on either the riverside or by the sea.

Pulmonary MAC infection and NRAMP1 *polymorphisms*

The results of case–control studies in patients with pulmonary MAC infection at four polymorphic loci of *NRAMP1* are shown

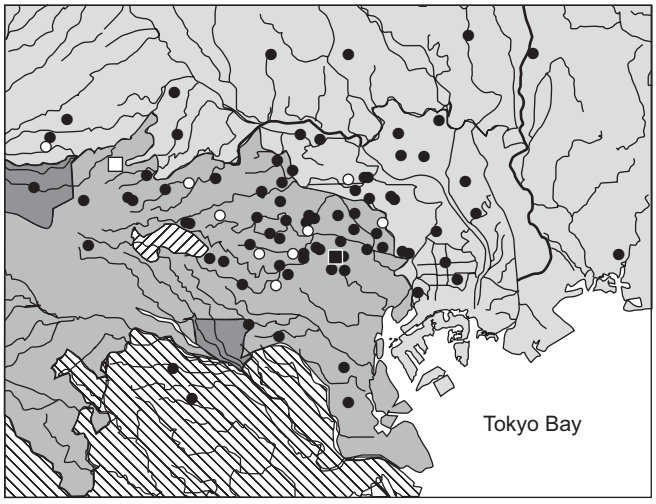

FIGURE 1. Residence at onset of pulmonary *Mycobacterium avium* complex (MAC) infection. The points where the first signs and symptoms of *Mycobacterium avium* (●) and *M. intracellulare* (○) infection were plotted on a regional map of Tokyo, with water systems in Tokyo superimposed. ■: International Medical Center of Japan; □: National Hospital Organization Tokyo Hospital; ■: Tonegawa river water system; ■: Tamagawa river water system; ■: combination of Tonegawa and Tamagawa water systems; ▨: Sagamigawa river water system; ▨: other river water systems.

in table 2. The genotypic distribution of the D543N and TGTG insertion/deletion polymorphism was significantly different between MAC cases and controls (p=0.026 and p=0.013, respectively). Homozygotes for major alleles were more often observed in MAC cases than in controls. A similar association was observed in the analysis with the second set of controls as shown in table 2 (p=0.003). A possible combined effect of the INT4 and 3′UTR polymorphisms, as reported previously [12], was also analysed. The overall comparison of combined genotypes did not show an association (p=0.107; data not shown). By haplotype analysis, there was no significant association between the INT4 and 3′UTR haplotypes and the pulmonary MAC infection (p=0.056; data not shown). The presence of the haplotype carrying the INT4-C allele and 3′UTR-del allele was not estimated in the present subjects (data not shown).

NRAMP1 *D543N polymorphism and subgroups of pulmonary MAC infection*

Differences of genotypic distribution of D543N and TGTG insertion/deletion polymorphism between cases and controls led to the analysis of possible differences between subgroups of MAC cases and controls. The TGTG insertion/deletion polymorphism was in perfect linkage disequilibrium with D543N and the genotype distributions of D543N described in table 3 are also representative of the TGTG polymorphism. The D543N polymorphism showed significant associations with MAC infection without predisposing conditions or when the main lesion was limited to the right middle lobe or lingular segment of the lung (p=0.009 and p=0.015, respectively). Such associations were obtained when each group was compared with control 2 as well as control 1 (p=0.006 and p=0.009, respectively). In contrast, the subgroup with comorbidity or when the main lesion was neither in the right middle lobe nor

TABLE 2 Relationship between the human natural resistance-associated macrophage protein 1 polymorphisms and pulmonary *Mycobacterium avium* complex (MAC) infection among the Japanese

Polymorphism	Patients with MAC	Control 1	Control 2	OR		p-value	
				Versus control 1	*Versus* control 2	*Versus* control 1	*Versus* control 2
Subjects n	111	177	247				
5′(GT)n							
Allele 1/allele 1	68 (61.3)	109 (61.6)	ND	1.0	ND	0.488	ND
Allele 1/allele 2	22 (19.8)	42 (23.7)	ND	0.84 (0.44-1.58)	ND		
Allele 1/allele 3	18 (16.2)	21 (11.9)	ND	1.37 (0.64-2.92)	ND		
Allele 2/allele 2	2 (1.8)	2 (1.1)	ND	1.60 (0.11-22.54)	ND		
Allele 2/allele 3	0 (0.0)	2 (1.1)	ND	0.00 (0.00-8.70)	ND		
Allele 3/allele 3	1 (0.9)	1 (0.6)	ND	1.60 (0.02-126.84)	ND		
INT4							
G/G	85 (76.6)	131 (74.0)	ND	1.0	ND	0.856	ND
G/C	25 (22.5)	44 (24.9)	ND	0.88 (0.50-1.54)	ND		
C/C	1 (0.9)	2 (1.1)	ND	0.77 (0.07-8.63)	ND		
D543N							
G/G	101 (91.0)	142 (80.2)	193 (78.1)	1.0	1.0	0.026	0.003
G/A	9 (8.1)	34 (19.2)	52 (21.1)	0.37 (0.17–0.81)	0.33 (0.16–0.70)		
A/A	1 (0.9)	1 (0.6)	2 (0.8)	1.41 (0.09–22.74)	0.96 (0.09–10.67)		
3′UTR							
TGTG ins/ins	101 (91.0)	141 (79.7)	193 (78.1)	1.0	1.0	0.013	0.003
TGTG ins/del	9 (8.1)	35 (19.8)	52 (21.1)	0.36 (0.17–0.78)	0.33 (0.16–0.70)		
TGTG del/del	1 (0.9)	1 (0.6)	2 (0.8)	1.40 (0.09–22.58)	0.96 (0.09–10.67)		

Data are presented as n (%) or mean (95% confidence interval), unless otherwise stated. Odds ratios (OR) represent the comparison between the most common homozygous genotype for each polymorphism. UTR: untranslated region; ins: insertion; del: deletion; ND: not done.

the lingular segment, failed to show significant associations with this genotype, although the number of patients are comparable to the other subgroup. Genotypic distribution of these alleles shown in the control is similar to that reported in other Japanese studies [28, 29].

Pulmonary MAC infection and VDR *polymorphisms*

Two polymorphisms of the *VDR* gene, the *Fok* I polymorphism in exon 3 and *Taq* I polymorphism in exon 10, were also analysed. Distributions of both polymorphisms were not different between MAC cases and controls (table 4).

Pulmonary MAC infection and MBL *polymorphisms*

Two polymorphisms of the *MBL* gene, -221 X/Y and codon 54 A/B polymorphisms were also tested. The distribution of both polymorphisms was not different between MAC cases and controls (table 5).

DISCUSSION

In general, interaction among pathogens, host factors and transmission routes are considered important for the development of infectious diseases. Although one report suggested that a familial aggregation of the disease is not caused by a

TABLE 3 Human natural resistance-associated macrophage protein 1 D543N polymorphism and subgroups of patients with pulmonary *Mycobacterium avium* complex infection

Genotype	MAC with comorbidity	p-value		MAC without comorbidity	p-value		RML/ lingular	p-value		Others	p-value	
		versus control 1	*versus* control 2		*versus* control 1	*versus* control 2		*versus* control 1	*versus* control 2		*versus* control 1	*versus* control 2
Subjects n	53			58			45			66		
D543N												
G/G	47 (88.7)	0.399	0.213	54 (93.1)	0.009	0.006	42 (93.4)	0.015	0.009	59 (89.4)	0.212	0.114
G/A	6 (11.3)			3 (5.2)			2 (4.4)			7 (10.6)		
A/A	0 (0.0)			1 (1.7)			1 (2.2)			0 (0.0)		

Data are presented as n or n (%), unless otherwise stated. RML: right middle lobe.

TABLE 4 Relationship between vitamin D receptor polymorphisms and pulmonary *Mycobacterium avium* complex (MAC) infection among the Japanese

Polymorphism	Patients with MAC	Controls	OR	p-value
Subjects	111	177		
***Fok* I**				
F/F	43 (39.1)	84 (47.5)	1.0	0.360
F/f	49 (44.5)	70 (39.5)	1.37 (0.82–2.30)	
f/f	18 (16.4)	23 (13.0)	1.53 (0.75–3.14)	
***Taq* I**				
T/T	87 (79.1)	132 (74.6)	1.0	0.635
T/t	22 (20.0)	41 (23.2)	0.81 (0.45–1.46)	
t/t	1 (0.9)	4 (2.2)	0.38 (0.04–3.45)	

Data are presented as n, n (%) or mean (95% confidence interval). Odds ratios (OR) represent comparisons between the most common homozygous genotype for each polymorphism.

TABLE 5 Relationship between mannose binding lectin polymorphisms and pulmonary *Mycobacterium avium* complex (MAC) infection among the Japanese

Polymorphism	Patients with MAC	Controls	OR	p-value
Subjects	111	177		
-221				
X/X	1 (0.9)	5 (2.8)	0.30 (0.04–2.65)	0.570
X/Y	20 (18.0)	35 (19.8)	0.87 (0.47–1.60)	
Y/Y	90 (81.1)	137 (77.4)	1.0	
Codon 54				
A/A	73 (65.8)	115 (65.0)	1.0	0.790
A/B	34 (30.6)	52 (29.4)	1.03 (0.61–1.74)	
B/B	4 (3.6)	10 (5.6)	0.63 (0.19–2.08)	

Data are presented as n, n (%) or mean (95% confidence interval). Odds ratios (ORs) represent comparisons between the most common homozygous genotype for each polymorphism.

single source of a particular virulent strain [10], molecular genetics of MAC causing pulmonary disease is rather limited.

When the mode of transmission of MAC is considered, a hospital water system or home water supply could represent a risk of MAC infection [2]. One study in South-East America [1] demonstrated that natural waters might be a source of pathogenic mycobacteria that can be transferred from water to air. The current authors plotted the place of residence of the subjects at the onset of the disease. Although this is not an all-embracing cohort study, it may be possible to conclude that the distribution of subjects' residence is not concentrated in a specific city water system or waterfront.

Genetic predisposition would be involved in the development of pulmonary MAC infection for the following reasons. In the past, mutational defects of several genes were identified as a cause of congenital cellular immune deficiency in several families of a disseminated form of MAC infection [30]. Furthermore, two studies showed that the human leukocyte antigen (HLA) *DR6* allele, encoded by the *HLA-DRB1* gene, or an Asian HLA haplotype including *DR6* is associated with sporadic cases of pulmonary MAC infection in the Japanese population [31, 32].

On the basis of strong influence of a mutation in the *NRAMP1* gene on susceptibility to intracellular pathogens in mice, there has been considerable interest in the relevance of the human homologue, *NRAMP1*, in susceptibility to human mycobacterial infection [12–16]. With regard to the MAC infection, there are only a few reports with small sample sizes. HUANG *et al.* [17] analysed the 5′(GT)n, D543N and 3′UTR insertion/deletion polymorphisms of the *NRAMP1* gene in eight patients with MAC and were unable to find any particular characteristics of allele patterns. TANAKA *et al.* [10] analysed the coding region of the *NRAMP1* gene in two Japanese families with pulmonary MAC infection, but were unsuccessful in finding any obvious abnormalities indicating immune deficiency. More recently, KOH *et al.* [18] reported a strong association between nontuberculous mycobacterial (NTM) lung disease including 18 patients with MAC infection and polymorphisms of the *NRAMP1* gene in a Korean population. However, this data should be interpreted with care, as it is estimated that at least seven (17.1%) of the 41 NTM patients possessed the 543-D and 3′UTR TGTG deletion haplotype, which had been thought to be rare in Asians [13, 16, 21, 33], including Korean TB patients [14]. Some factors unique to their study population, such as population stratification, might also have had an effect on the results, while the authors described uniformity of ethnicity.

To the current authors' knowledge, this is the first large-scale study in which >100 blood samples were collected for analysis of the candidate genes for pulmonary MAC infection. To exclude the possibility of false positives owing to a biased genotypic distribution of the controls, another set of controls were also tested and again significant association was observed. The allele frequency of the 543-N and TGTG deletion of 3′UTR were significantly lower in the MAC group than in controls. Although the amino acid change in the coding region and 3′UTR polymorphism of the *NRAMP1* gene might influence the function and mRNA levels of the gene, respectively, the functional significance of these polymorphisms has not yet been investigated extensively. It is not surprising that the (GT)n promoter polymorphism of the *NRAMP1* gene was not associated with MAC infection in the present study, as earlier studies analysing genomic structure around the *NRAMP1* gene demonstrated that linkage disequilibrium is not strong enough between the 5′ and 3′ end of the gene [12, 21, 33], namely the (GT)n repeats and D543N/TGTG polymorphisms, although the D543N and TGTG insertion/deletion polymorphism are in perfect linkage disequilibrium. It is interesting that in a West African population, the same 3′UTR deletion allele was similarly low in subjects with a paucibacillary tuberculoid form of leprosy, where a

T-helper cell (Th)1-type response is more predominant, than in multibacillary form, which is associated with a Th2-type immune response [15]. Conversely, however, the study by BELLAMY *et al.* [12] clearly showed that all four polymorphisms were associated with smear-positive TB and the 543-N and TGTG deletion showed a more positive association with TB in Gambians, whereas, in a Cambodian population, the 543-N and TGTG deletion showed a negative association with active TB [16]. Apparently divergent findings may be explained by the presence of another possible susceptibility variant [33]. In the study by BELLAMY *et al.* [12], a strong association was demonstrated by combined analysis of the INT4 and 3′UTR variants. However, in the current study, the GC/+del allele did not increase the risk for development of pulmonary MAC disease. The haplotype carrying the INT4-C allele and 3′UTR-del allele, which were both reported as susceptible alleles in the study of BELLAMY *et al*. [12], might not exist in the Japanese population according to the current frequency estimation. This might partly explain the differences between the present study and the report from an African population [12].

The present authors classified patients into two groups, with or without predisposing risk factors, as described in the 1997 ATS statement [3]. Most MAC patients without a predisposing condition were elderly females, and the right middle lobe or lingular segment of the lung was mainly affected. This appears to be one of the characteristics of pulmonary MAC infection without comorbidity [6–8]. Although the mechanism is unknown, changes in hormonal balance accompanied with ageing might be involved in the development of the disease [34]. The *NRAMP1* polymorphism showed a relatively strong association with MAC infection where there is no predisposing condition or in cases where the main lesion is limited to the right middle lobe or lingular segment of the lung. In contrast, the subgroup without this phenotype did not show significant associations. It is consistent with the notion that a certain genetic predisposition underlies this particular phenotype in affected individuals. When the results of previous and future studies on MAC infection are assessed, this phenotypic effect should be taken into account.

CONCLUSION

The current authors investigated the human natural resistance-associated macrophage protein 1, vitamin D receptor and mannose binding lectin gene polymorphisms, previously reported as candidate genes determining susceptibility to mycobacterial infection, and demonstrated a possible influence of the natural resistance-associated macrophage protein 1 polymorphisms on the development of pulmonary *Mycobacterium avium* complex infection. Possible genetic risk factors that permit infection with *Mycobacterium avium* complex in otherwise normal individuals could be targeted for future therapeutic intervention.

ACKNOWLEDGEMENTS

The authors wish to thank: A. Mikami, S. Izumi, M. Kamimura, H. Kawada and A. Yoshizawa (International Medical Center of Japan, Tokyo, Japan), and Y. Kawabe, K. Masuda, M. Nishiyama and Y. Tanaka (National Hospital Organization Tokyo Hospital, Tokyo, Japan) for making the patients under their care available; M. Okochi (International Medical Center of Japan) for her technical assistance; and K. Tanabe (International Medical Center of Japan) for critical reading the manuscript. The authors would especially like to thank S. Akagawa and N. Nagayama (National Hospital Organization Tokyo Hospital) for their continuous encouragement.

REFERENCES

1 Wendt SL, George KL, Parker BC, Gruft H, Falkinham JO 3rd. Epidemiology of infection by nontuberculous Mycobacteria. III. Isolation of potentially pathogenic mycobacteria from aerosols. *Am Rev Respir Dis* 1980; 122: 259–263.

2 du Moulin GC, Stottmeier KD, Pelletier PA, Tsang AY, Hedley-Whyte J. Concentration of *Mycobacterium avium* by hospital hot water systems. *JAMA* 1988; 260: 1599–1601.

3 Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Medical Section of the American Lung Association. *Am J Respir Crit Care Med* 1997; 156: S1–S25.

4 Sakatani M. Nontuberculous mycobacteriosis; the present status of epidemiology and clinical studies. *Kekkaku* 1999; 74: 377–384.

5 Henry MT, Inamdar L, O'Riordain D, Schweiger M, Watson JP. Nontuberculous mycobacteria in non-HIV patients: epidemiology, treatment and response. *Eur Respir J* 2004; 23: 741–746.

6 Prince DS, Peterson DD, Steiner RM, *et al*. Infection with *Mycobacterium avium* complex in patients without predisposing conditions. *N Engl J Med* 1989; 321: 863–868.

7 Reich JM, Johnson RE. *Mycobacterium avium* complex pulmonary disease. Incidence, presentation and response to therapy in a community setting. *Am Rev Respir Dis* 1991; 143: 1381–1385.

8 Reich JM, Johnson RE. *Mycobacterium avium* complex pulmonary disease presenting as an isolated lingular or middle lobe pattern. The Lady Windermere syndrome. *Chest* 1992; 101: 1605–1609.

9 Tanaka E, Amitani R, Niimi A, Suzuki K, Murayama T, Kuze F. Yield of computed tomography and bronchoscopy for the diagnosis of *Mycobacterium avium* complex pulmonary disease. *Am J Respir Crit Care Med* 1997; 155: 2041–2046.

10 Tanaka E, Kimoto T, Matsumoto H, *et al*. Familial pulmonary *Mycobacterium avium* complex disease. *Am J Respir Crit Care Med* 2000; 161: 1643–1647.

11 Vidal SM, Malo D, Vogan K, Skamene E, Gros P. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. *Cell* 1993; 73: 469–485.

12 Bellamy R, Ruwende C, Corrah T, McAdam KP, Whittle HC, Hill AV. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. *N Engl J Med* 1998; 338: 640–644.

13 Gao PS, Fujishima S, Mao XQ, *et al*. Genetic variants of NRAMP1 and active tuberculosis in Japanese populations. International Tuberculosis Genetics Team. *Clin Genet* 2000; 58: 74–76.

14 Ryu S, Park YK, Bai GH, Kim SJ, Park SN, Kang S. 3′UTR polymorphisms in the NRAMP1 gene are associated with susceptibility to tuberculosis in Koreans. *Int J Tuberc Lung Dis* 2000; 4: 577–580.

15 Meisner SJ, Mucklow S, Warner G, Sow SO, Lienhardt C, Hill AV. Association of NRAMP1 polymorphism with leprosy type but not susceptibility to leprosy *per se* in west Africans. *Am J Trop Med Hyg* 2001; 65: 733–735.

16 Delgado JC, Baena A, Thim S, Goldfeld AE. Ethnic-specific genetic associations with pulmonary tuberculosis. *J Infect Dis* 2002; 186: 1463–1468.

17 Huang JH, Oefner PJ, Adi V, *et al.* Analyses of the NRAMP1 and IFN-gammaR1 genes in women with *Mycobacterium avium-intracellulare* pulmonary disease. *Am J Respir Crit Care Med* 1998; 157: 377–381.

18 Koh WJ, Kwon OJ, Kim EJ, Lee KS, Ki CS, Kim JW. NRAMP1 gene polymorphism and susceptibility to non-tuberculous mycobacterial lung diseases. *Chest* 2005; 128: 94–101.

19 Bellamy R, Ruwende C, Corrah T, *et al.* Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. *J Infect Dis* 1999; 179: 721–724.

20 Soborg C, Madsen HO, Andersen AB, Lillebaek T, Kok-Jensen A, Garred P. Mannose-binding lectin polymorphisms in clinical tuberculosis. *J Infect Dis* 2003; 188: 777–782.

21 Liu J, Fujiwara TM, Buu NT, *et al.* Identification of polymorphisms and sequence variants in the human homologue of the mouse natural resistance-associated macrophage protein gene. *Am J Hum Genet* 1995; 56: 845–853.

22 Brownstein MJ, Carpten JD, Smith JR. Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques* 1996; 20: 1004–1006.

23 Curran JE, Vaughan T, Lea RA, Weinstein SR, Morrison NA, Griffiths LR. Association of A vitamin D receptor polymorphism with sporadic breast cancer development. *Int J Cancer* 1999; 83: 723–726.

24 Lipscombe RJ, Beatty DW, Ganczakowski M, *et al.* Mutations in the human mannose-binding protein gene: frequencies in several population groups. *Eur J Hum Genet* 1996; 4: 13–19.

25 The R project for statistical computing. http://www.r-project.org. Date last updated: March 2003. Date last accessed: February 24, 2006.

26 Burkett K, McNeney B, Graham J. A note on inference of trait associations with SNP haplotypes and other attributes in generalized linear models. *Hum Hered* 2004; 57: 200–206.

27 The Bureau of Waterworks, Tokyo Metropolitan Government. Water Resources and Supply Areas. http://www.waterworks.metro.tokyo.jp. Date last accessed: August 28, 2005.

28 Ouchi K, Suzuki Y, Shirakawa T, Kishi F. Polymorphism of SLC11A1 (formerly NRAMP1) gene confers susceptibility to Kawasaki disease. *J Infect Dis* 2003; 187: 326–329.

29 Abe T, Iinuma Y, Ando M, *et al.* NRAMP1 polymorphisms, susceptibility and clinical features of tuberculosis. *J Infect* 2003; 46: 215–220.

30 Ottenhoff TH, Verreck FA, Lichtenauer-Kaligis EG, Hoeve MA, Sanal O, van Dissel JT. Genetics, cytokines and human infectious disease: lessons from weakly pathogenic mycobacteria and salmonellae. *Nat Genet* 2002; 32: 97–105.

31 Kubo K, Yamazaki Y, Hanaoka M, *et al.* Analysis of HLA antigens in *Mycobacterium avium-intracellulare* pulmonary infection. *Am J Respir Crit Care Med* 2000; 161: 1368–1371.

32 Takahashi M, Ishizaka A, Nakamura H, *et al.* Specific HLA in pulmonary MAC infection in a Japanese population. *Am J Respir Crit Care Med* 2000; 162: 316–318.

33 Yip SP, Leung KH, Lin CK. Extent and distribution of linkage disequilibrium around the SLC11A1 locus. *Genes Immun* 2003; 4: 212–221.

34 Tsuyuguchi K, Suzuki K, Matsumoto H, Tanaka E, Amitani R, Kuze F. Effect of oestrogen on *Mycobacterium avium* complex pulmonary infection in mice. *Clin Exp Immunol* 2001; 123: 428–434.