Recent advances in the molecular epidemiology of leprosy

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Recent advances in the molecular epidemiology of leprosy through genotyping of variable number tandem repeats (VNTRs) and single nucleotide polymorphisms (SNPs) are described. VNTRs with a broad range of diversity are useful genotyping tools for analyzing transmission in community areas, and SNPs and VNTRs with a small degree of variation are favorable for investigating the global transmission of leprosy. We expect that the transmission of leprosy can be fully analyzed by the application of these new methodologies.

Introduction

The number of newly detected cases of leprosy has been gradually decreasing around the world in recent years ¹⁾. Even so, some details of disease transmission remain to be elucidated. One of the important subjects still under dispute, and essential for establishing a better strategy for preventing new cases, is the transmission mode of leprosy including the infectious source. Phenotype, serotype, phage type and genotype are robust metrics for analyzing and tracing transmission in many infectious diseases ^{2, 3)}. These useful tools were not available

for understanding the transmission of leprosy until two kinds of variable number tandem repeat (VNTR) were identified ^{4, 5)}, followed by the discovery of single nucleotide polymorphisms (SNPs) in the *Mycobacterium leprae* genome ⁶⁾. Epidemiological studies of leprosy using these two assays have been gradually accumulating since 2004, and findings generated by these breakthroughs in molecular epidemiology are now taking the place of classical epidemiological methods. An international study group for investigating the transmission of leprosy through the application of advances in molecular biology, including genotyping, has been organized and collaborative studies have begun ⁷⁾. VNTRs with broad allelic diversity are useful for analyzing transmission patterns in the community ⁸⁻¹¹⁾. SNPs and VNTRs with a narrow range of diversity are practical for characterizing the global geographical distribution of different genotypes of *M. leprae*,

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and are beneficial in tracing the dissemination of leprosy in prehistoric times and following current global leprosy migration patterns¹²⁻¹⁶. This article describes recent advances in the molecular epidemiology of leprosy.

Application of VNTRs to Analyze the Transmission of Leprosy

It has long been believed that leprosy is transmitted by heavy contact with leprosy patients; however, there are some findings which contradict this premise. For instance, a study on the geographical distribution of responders to *M. leprae* antibodies showed a distribution of positive responders in houses remote from those with known patients¹⁷. The limited effect of chemoprophylaxis¹⁸ means that drugs prevent the progression of an active infection to overt disease¹⁹ but do not interrupt transmission. Attempts to curtail transmission and reduce new infections by chemoprophylaxis in the Federated States of Micronesia (FMS) failed. The number of new cases detected in FMS has increased again after 1999 and over the last several years has risen to nearly the same level as before execution of the chemoprophylaxis program¹. Many new cases without known contact with leprosy patients^{20, 21} have also been reported. These findings suggest gaps in the traditional concept of leprosy transmission and imply the existence of an infectious source of *M. leprae* other than leprosy patients.

PCR studies have shown that in areas with a high prevalence of leprosy, many individuals harbor *M. leprae* in their nasal mucus^{22, 23}. These findings indicate that people in such areas are frequently exposed to *M. leprae* infection. The hypothesis that the bacterial genotypes from household members or other contacts of patients should be identical if the bacilli are transmitted by contact

with the patient, was tested by a comparison of genotypes from the nasal mucus of patients and family members, and also from new patients and the supposed index case of a multifamily case⁸. Genotyping based on the copy number of a TTC repeat^{5, 8} and another genotyping method with higher discriminatory power were applied¹⁰. Results revealed that the genotype of *M. leprae* in the nasal mucus differed between the patient and contacts in a given household. The existence of *M. leprae* strains with allelic diversity in a multifamily case in China was also shown¹¹. On the other hand, a study conducted in India revealed no divergence among the three VNTRs from two patients in a multifamily case⁹. The lack of divergence among the patients of this case could be explained by the low discriminatory power of genotyping using only three polymorphic VNTRs. In another study, isolates determined as having identical genotypes using a few VNTRs were separated into different genotypes by a method with high resolution power using a combination of other appropriate VNTRs¹⁰. Thus, we theorize that the Indian clinical isolates with identical genotypes might be classified into different genotypes with the improved methodology. Although VNTRs seem to fluctuate in biopsy samples from the same patient^{24, 25}, co-infection of *M. leprae* strains with different VNTRs may occur. While the variation of VNTRs in a given patient is often by only one or two copies, the TTC copy number of the isolate from a supposed index case was 18 and the TTC copy number of the bacilli from his son was 10 in one multifamily case report⁸. It is clear that the genotypes of the bacilli from father and son were distinct and hence did not come from the same infectious source⁸. This suggests the presence of an alternate source of infection.

Water sources used daily by people in an endemic area are thought to be a potential source

of *M. leprae* infection, since many water-borne mycobacterial infectious diseases exist^{26, 27, 28)}. Water samples collected at 6 villages with a high prevalence of leprosy in Indonesia were assayed by PCR. Of 44 water samples, 21 were positive by PCR assay for a 65 kDa protein coding sequence²⁹⁾. The relative risk of leprosy infection was 3.24 for those using the PCR-positive water source for washing and bathing, but no difference was shown in risk for water used for drinking and cooking. Water is heated for drinking and cooking, and consequently bacilli in the water are inactivated²⁹⁾. PCR positivity does not prove the existence of live *M. leprae* in the water. However, reverse transcription-PCR (RT-PCR) targeting 16s rRNA did detect the presence of *M. leprae* organisms³⁰⁾. This method was used to analyze the water from wells in a village on the Indonesian island of North Sulawesi that has many newly detected patients every year. Of 51 well water samples, 17 were positive by RT-PCR³¹⁾. These results strongly suggest that water is an environmental reservoir of *M. leprae* and an infectious source of leprosy. Possible infection from the soil has also not been ruled out³²⁾.

Application of SNPs and VNTRs with a Small Range of Diversity to Analyze the Global Dissemination of Leprosy

Using a VNTR with either 3 or 4 copies of a 6-bp sequence, a pattern in the global geographical distribution of *M. leprae* has been found^{4, 12, 13, 15, 16)}. The 3-copy strain is overwhelmingly predominant in most countries around the world, while the 4-copy strain is predominantly found in East Asia, including the mainland of Japan, Korea and eastern parts of China, as well as western parts of Mexico^{4, 12-14)}. No 4-copy isolates have been obtained from the Okinawa and Amami Islands

of Japan. These islands are located at the southern end of Japan and have their own unique culture. The presence of different genotypes between these two regions of Japan is also found in other species of microorganisms^{33, 34)}. It is thought that the predominance of the 4-copy strain in East Asian countries was established in prehistoric times by the movement of Mongoloid people in the region. In Japan, it is likely that the characteristic distribution of each genotype was established by the movement of two ethnic groups which migrated to Japan about 30,000 years ago through the Okinawa Islands, and 2,800 to 1,700 years ago through the Korean peninsula³⁵⁾. Specifically, *M. leprae* of the 4-copy strain might have been carried by the people who migrated to Japan through the Korean Peninsula, while the 3-copy strain was spread to the Okinawa Islands by the people who migrated to Japan from Southeast Asia 30,000 years ago. This example demonstrates how VNTRs with small range of polymorphic variation, such as that of the *rpoT* gene, are useful in tracing the global transmission of leprosy.

The geographical distribution of *M. leprae* SNP alleles 1-4 is also suitable for analyzing the global spread of leprosy⁶⁾. An assay using a combination of SNPs and the *rpoT* gene VNTR was applied to determine whether Japanese Brazilian patients newly diagnosed in the mainland of Japan had been infected in Japan or Brazil¹⁴⁾. SNP type 4, detected in two Japanese Brazilian patients, was not found among 46 Japanese patients from the mainland. The isolate with SNP type 3 and the 3-copy polymorphism of the *rpoT* gene, found in 7 isolates from Japanese Brazilian cases, is also uncommon in mainland Japan. Thus it is most likely that the patients were infected in Brazil and developed symptoms of leprosy after they came to Japan. It had been generally assumed that this was the case since the prevalence of leprosy is high in

Brazil, but there was no substantial microbiological evidence to support the idea. The assay combining SNPs and the *rpoT* gene VNTR clearly contributed to the analysis of the global transfer of leprosy incident to the current movement of people.

The distribution of each SNP genotype in Asian countries was also examined in another study ¹⁴⁾. Most of the isolates (26 of 29) from Myanmar had SNP type 1. The frequency of this SNP declines in proportion to the distance of the countries from India, as is seen by low proportions in Indonesia, Korea and Japan. Logically, the SNP type 2 isolate could be derived from a type 1 progenitor, followed by evolution to type 3 and type 4 derivatives. All isolates from the southern part of India had SNP type 1 ⁶⁾. Taken together with data from other studies, it seems likely that leprosy originated in the Indian subcontinent and spread outward to other areas of the world. This idea is compatible with the argument that leprosy originated in the Indian subcontinent and was introduced to Egypt as a result of Persian invasion during the sixth century BC or by the troops of Alexander the Great in the fourth century BC ³⁶⁾.

The use of molecular epidemiology through the application of genotyping as a means of tracking the transmission of leprosy began just 4 years ago in 2004, on the basis of a milestone discovery of two VNTRs in the genomic DNA of *M. leprae*. This new field of leprosy research is expected to illuminate obscure mechanisms of leprosy transmission with respect to sources, reservoirs, modes of transmission and the history of leprosy.

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ハンセン病の分子疫学解析における最近の知見

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キーワード：ハンセン病、分子疫学、らい菌、SNPs、VNTRs

らい菌遺伝子の直列繰返配列多型（variable number tandem repeat：VNTRs）及び 1 塩基多型（single nucleotide polymorphism: SNPs）を用いたハンセン病の分子疫学の最近の知見が述べられた。VNTRs は地域社会を対象としたハンセン病の伝搬の解析にまた、SNPs 並びに多様性の小さな VNTRs はハンセン病の地球規模での伝搬の解析に有用であることが述べられた。これらの手技をもちいることにより、ハンセン病の感染様式がより明確にされることが期待される。

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