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# Genotyping of *Giardia* Isolates from Humans in Japan Using the Small Subunit Ribosomal RNA and Glutamate Dehydrogenase Gene Sequences

Niichiro Abe\*, Isao Kimata<sup>1</sup> and Masaharu Tokoro<sup>2</sup>

Department of Microbiology, Osaka City Institute of Public Health and Environmental Sciences, Osaka 543-0026,

<sup>1</sup>Department of Protozoal Diseases, Graduate School of Medicine, Osaka City University, Osaka 545-8585 and

<sup>2</sup>Department of Parasitology, Graduate School of Medical Science, Kanazawa University, Kanazawa 920-8640

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The flagellate *Giardia intestinalis* (syn. *G. lamblia*, *G. duodenalis*) is a well-known intestinal parasite which causes enteric diseases in humans, livestock, and companion animals. Recent molecular studies have shown that *G. intestinalis* is composed of at least seven genetically distinct but morphologically identical assemblages (Assemblages A to G), and that most of these assemblages appear to have different host preferences, e.g., Assemblages C and D are found in dogs, Assemblage E in hoofed livestock, Assemblage F in cats, and Assemblage G in rats (1). Assemblage A, however, consists of isolates that can be classified into two genetic groups (1): genetic group A-I is isolated from a variety of animals including humans, while Assemblage A-II is isolated exclusively from humans. Assemblage B consists of a genetically diverse group of mostly human isolates, but some isolates from animals are included. Thus, the *G. intestinalis* isolates that have the potential for zoonotic transmission seem to be restricted within narrow genetic groups, specifically Assemblages A and B (1).

In Japan, giardiasis has been classified as a category V notifiable infectious disease in the National Epidemiological Surveillance of Infectious Diseases under the Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections enacted in April of 1999. Although approximately one hundred cases of this infection were reported annually between 2000 and 2004 (<http://idsc.nih.gov/jp/iasr/virus/virus-e.html>), the molecular epidemiology of *Giardia* in Japan remains unclear. To date, only two human isolates have been genotyped as Assemblage B in Japan (2,3). In the present study, we genotyped three isolates of *G. intestinalis* from humans in Japan using both small subunit ribosomal RNA and glutamate dehydrogenase gene sequences.

The three isolates (GH-125, GH-126 and GH-135) examined in the present study were isolated from Japanese individuals: isolates GH-125 and GH-126 were from asymptomatic individuals living in Osaka, and GH-135 came from a diarrheal HIV-positive patient in Tokyo. *Giardia* cysts were purified from each fecal sample by the sucrose centrifugal flotation method (4), and the genomic DNA was extracted and purified following the method reported previously (4,5).

*Giardia* diagnostic fragments were amplified by polymerase chain reaction (PCR) with the following primer pairs targeting the different gene loci: RH11 and RH4 for the *Giardia* small subunit ribosomal RNA gene (SSUrDNA) (6) and GDH1 and GDH4 for the *Giardia* glutamate dehydrogenase gene (GDH) (7). PCR amplification of SSUrDNA was performed using LA Taq polymerase with 2X GC buffer I (LA Taq) (TaKaRa Shuzo Co., Ltd., Otsu, Japan), and amplification of GDH using Ex Taq polymerase with 10X Ex Taq buffer (Ex Taq) (TaKaRa Shuzo) as reported previously (5). Sequencing of the PCR products and phylogenetic analysis were performed following the methods reported previously (2,8). The partial sequences of the SSUrDNA and GDH of each isolate were deposited in the GenBank database under accession numbers AB195219-AB195224.

SSUrDNA and GDH were successfully amplified in all isolates examined in the present study (data not shown). Partial sequences of the SSUrDNA of GH-125 and GH-126 were found to be identical to those of BAH40C11 and BAC2 that are known to belong to Assemblage A. Similarly, GH-135 had a sequence identical to those of BAH-12 and Ad-28 in Assemblage B (Fig. 1A). More precisely, analysis of GDH partial sequences (592 bp) made it possible to distinguish GH-125, which had a sequence identical to those of Ad-2 and Bris-136, from GH-126 by 3 bp differences, even though they were both classified into the anthroponotic genotype Assemblage A-II (Fig. 1B). Again, the GDH partial sequence of GH-135 was almost identical to that of BAH-12 with 2 bp differences and was grouped into zoonotic Assemblage B (Fig. 1B).

Recently, two human isolates of *Giardia*, GH-156 and GH-158, were genotyped as Assemblage B by phylogenetic analysis using GDH partial sequences in Japan (2,3). In addition, three distinct genotypes, pertaining to Assemblages A-I, D and E, have been isolated from a ferret, dogs and calves, respectively (2,5,8). Genotypes of Assemblage A-I are known to have wider range of host species and have the potential to infect humans, while Assemblages D and E are known to be host-specific and non-infective to humans. Based on the results reported in the present experiment together with those reported elsewhere (2,3,8), there are three *Giardia* genotypes present in Japan that are either of zoonotic (Assemblage A-I and Assemblage B) or anthroponotic (Assemblage A-II) potential for human infection. Further genetic analysis of both human and animal isolates of this microbe is needed to gain greater insight into the molecular epidemiology of endemic *G. intestinalis* in Japan.

\*Corresponding author: Mailing address: Department of Microbiology, Osaka City Institute of Public Health and Environmental Sciences, 8-34 Tojo-cho, Tennoji-ku, Osaka 543-0026, Japan. Tel: +81-6-6771-3147, Fax: +81-6-6772-0676, E-mail: n.abe@iphes.city.osaka.jp

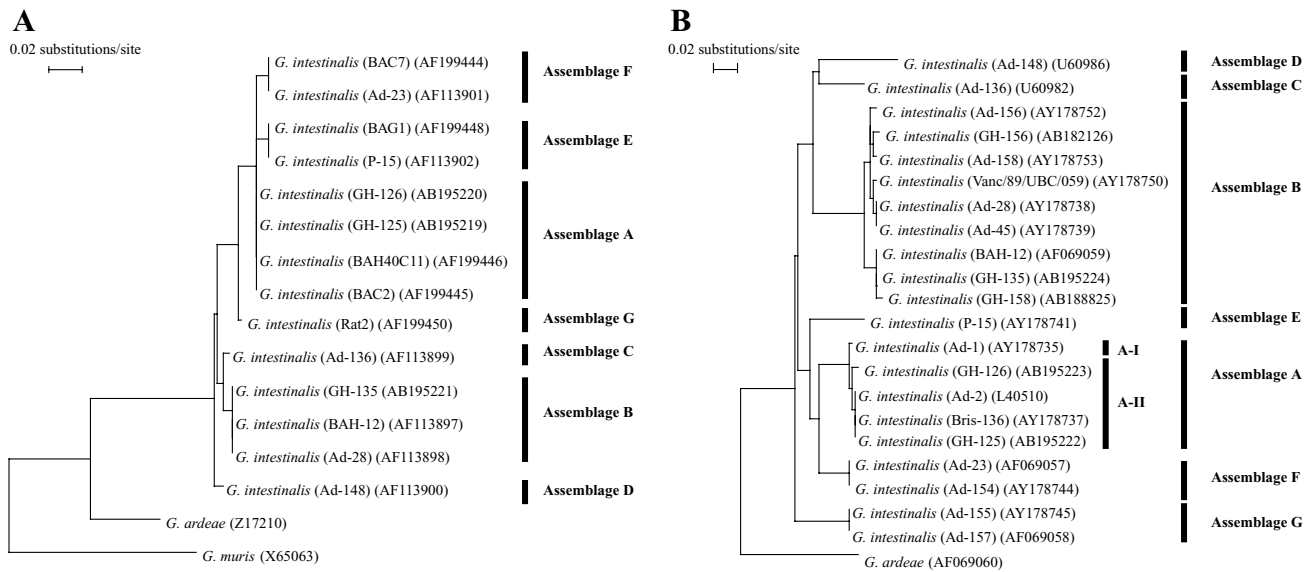


Fig. 1. Phylogenetic relationships of the isolates from humans examined in the present study to other *Giardia* spp. and *G. intestinalis* genotypes as inferred by neighbor-joining analysis, based on the nucleotide sequences of SSUrDNA (A) and GDH (B). Names of the isolates and accession numbers in GenBank are shown in parentheses.

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