**Pneumocystis jiroveci** Genotypes and Primary Infection

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This study describes the molecular typing of *Pneumocystis jiroveci* organisms from 5 nonpremature immunocompetent infants who developed a primary infection. Four *P. jiroveci* internal transcribed spacer (ITS) types were identified. All have been previously described in reports concerning immunosuppressed adults with pneumocystosis. Present data suggest that identical types can be implicated either in first contact or in additional contacts between fungus and host and that both immunocompetent infants and immunocompromised patients may be part of a common human reservoir for the fungus.

Seroepidemiological surveys conducted in the 1970s have indicated that humans commonly develop a primary *Pneumocystis* infection early in life [1]. It was recently shown that *Pneumocystis jiroveci* (human-derived *Pneumocystis*) can be detected in nasopharyngeal aspiration (NPA) specimens from immunocompetent infants at risk for primary infection [2, 3]. However, no data concerning the genomic characteristics of the fungus found in these infants is available yet, and genotyping of *P. jiroveci* has mostly been performed on isolates obtained from immunosuppressed patients who developed acute *Pneumocystis* pneumonia (PCP) [4, 5]. The evidence of shared *P. jiroveci* genotypes in the 2 patient populations, consistent with their both being implicated in a common human reservoir of the fungus, would be key information in *P. jiroveci* epidemiology.

In the present study, 5 archival *P. jiroveci* isolates obtained from 5 nonpremature immunocompetent infants who developed a primary *Pneumocystis* infection were examined for genotyping (table 1). Signed informed consent was obtained from the parents of children who participated. The infants from whom isolates were obtained were initially hospitalized in our institution (CHU d’Amiens, France) with clinical and radiological features related to a bronchiolitis episode. They underwent NPA to detect viruses, bacteria, and *P. jiroveci*. The fungus was detected in NPA specimens with a PCR assay directed at the mitochondrial large subunit rRNA (mtLSUrRNA) gene, as described elsewhere for immunosuppressed children who developed PCP [6, 7]. Serum samples obtained from infants were examined for antibodies against *Pneumocystis* by immunoblotting with use of a commercial monoclonal antibody to a *P. jiroveci* component (Dako) as a positive control. Immunoblotting results were negative for all infants. These results excluded a prior *Pneumocystis* infection and showed that the presence of *P. jiroveci* was related to a first contact.

The genotyping of *P. jiroveci* was based on sequence analysis of ITS 1 and ITS 2 regions of the nuclear ribosomal RNA operon, which are considered to be more informative than the mtLSUrRNA gene [4, 5]. Sequences were amplified using a nested PCR assay with 2 pairs of *P. jiroveci* specific primers, N18SF and N26SRX [8], and ITSF3 and ITS2R3 [4]. The products of the second PCR round were cloned into the plasmid vector pGEM-T, (pGEM-T Vector System II, Promega Corporation) and sequenced from the 2 strands (with use of the BigDye Terminators Method and the Applied Biosystems Sequencer 3700, Applied Biosystems). ITS 1 and ITS 2 alleles were subsequently identified using the 2 typing systems described by Tsolaki et al. [4] and Lee et al. [5], respectively. A *P. jiroveci* ITS type is defined by a combination of the two ITS 1 and ITS 2 alleles.

With the use of the typing system of Tsolaki et al. [4], four *P. jiroveci* ITS types were detected (table 1): type A6C4, in 2 infants, types B1b4, B1a4, and B1d4, in 1 infant each. These 4 types correspond to types Al or Bl, Eb, N6, and Ea, respectively, with use of the typing system of Lee et al. [5].

This work presents the first data concerning *P. jiroveci* ITS types in nonpremature immunocompetent infants who developed a primary infection. The diagnosis of primary infection was not based on seroconversion to *Pneumocystis* antibodies.
since the ethics committee of our institution did not approve any blood sampling from asymptomatic infants who had recovered after the hospitalization period. Therefore, diagnosis criteria consisted of association of an acute respiratory syndrome with the presence of *P. jiroveci*, with the simultaneous absence of serum anti-*Pneumocystis* antibodies excluding prior infection with the fungus.

For the 5 infants, clinical improvement was obtained with short-term hospitalization (1–6 days), even though they did not receive drugs active against the fungus. For this reason, *P. jiroveci* detection by PCR was not considered to reflect a severe infection. Furthermore, viruses or bacteria that were detected in association with *P. jiroveci* for 4 infants were either partly or totally responsible for respiratory symptoms. Therefore, primary infection presents as a mild *Pneumocystis* infection that can be contemporaneous with other respiratory infections.

Different *P. jiroveci* ITS types were identified in 5 infants, showing a high diversity of types. These types and a similar type diversity were previously detected among nonepidemiologically linked *P. jiroveci* isolates obtained from immunocompromised adults with PCP living in different regions of Europe and the United States [4, 5]. Therefore, identical *P. jiroveci* ITS types can be implicated either in first contact in infancy or in further contacts throughout life. These shared features of *P. jiroveci* ITS types in immunocompetent infants and immunosuppressed adults suggest that *P. jiroveci* acquisition may result from common sources.

It is now widely accepted that animal sources for *P. jiroveci* can be excluded because the *Pneumocystis* organisms that infect mammalian species are host specific [9]. Moreover, it has been suggested that *Pneumocystis* infections in humans are actively acquired from a common external source and that humans are important in the transmission cycle of *P. jiroveci* [10]. Observations suggest that interindividual transmission may occur [11], as demonstrated in rodent models [12]. Although an exosporophytic form of *P. jiroveci* cannot be strictly ruled out, these data suggest that humans are the main *P. jiroveci* infection sources and the main reservoir for the fungus.

Our results of genotyping in infants and the results of previous studies of adult patients with PCP [4, 5] are consistent with the hypothesis that both patient populations are part of a common human reservoir for *P. jiroveci*. These data, combined with data on the high frequency of primary *Pneumocystis* infection among infants [1], suggest that the infant population may represent an important part of this reservoir.

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**References**


