

## Absence of *Mycoplasma* species DNA in chronic fatigue syndrome

People with chronic fatigue syndrome (CFS) suffer from multiple symptoms that include fatigue, problems with memory, concentration, sleep and musculoskeletal pain. The association of any one infectious agent with CFS has been difficult to establish and is complicated further by the lack of a known lesion or diseased tissue to sample. Peripheral blood, by its nature of systemic circulation, serves as an ideal specimen in CFS, as it samples the entire body and could thus serve as a sentinel for diverse lesions. Plasma, the liquid portion of peripheral blood that is devoid of cells, is known to contain remnants of numerous physiological and disease processes. We used plasma DNA to detect and characterize bacterial 16S rDNA sequences in a group of individuals with CFS and a group of non-fatigued controls (Vernon *et al.*, 2002). Whilst a variety of bacterial sequences were detected in both fatigued and non-fatigued groups, no *Mycoplasma* sp. 16S rDNA sequences were found. A few studies have reported the detection of several *Mycoplasma* species in peripheral blood cells of CFS patients by using *Mycoplasma* species-specific PCR (Nasralla *et al.*, 1999; Nijs *et al.*, 2002). In this study, we used *Mycoplasma* species-specific primer pairs for direct amplification of these microbes from plasma DNA.

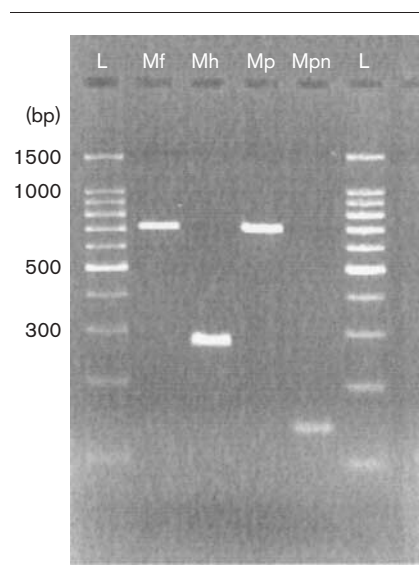
As part of a population-based study of CFS in Wichita, Kansas, USA (Reyes *et al.*, 2003), peripheral blood was collected during the clinical evaluation of 34 subjects, classified according to the 1994 international case definition for CFS, and a random selection of 55 non-fatigued subjects. Blood was collected in sodium citrate Vacutainer tubes (Beckton Dickinson) and shipped by overnight courier to the Centers for Disease Control (CDC), where plasma was collected by separation on lymphocyte separation medium (LSM; ICN Biomedicals). Plasma (1 ml) was concentrated to approximately 250 µl in a Centricon centrifugal filter unit YM-100 (Millipore). Cell-free plasma DNA was extracted by using a QIAamp DNA Mini kit (Qiagen) according to the manufacturer's instructions and quantified

by using a DyNA Quant 200 fluorometer (Amersham Biosciences).

Primer pairs for *Mycoplasma fermentans*, *Mycoplasma hominis*, *Mycoplasma penetrans* and *Mycoplasma pneumoniae* were synthesized according to published sequences. Conditions for each primer pair were optimized and shown to amplify 0.3–2 pg genomic equivalents of *Mycoplasma* DNA (Fig. 1). Despite these optimized conditions and the high level of sensitivity of this amplification method, no *Mycoplasma* products were amplified from any of the 89 cell-free plasma DNA samples.

There have been a few reports of an association between infection with *Mycoplasma* species and CFS. One study has shown *M. fermentans* to be the mycoplasma detected most commonly by PCR in the blood of CFS patients from the USA (Nasralla *et al.*, 1999). However, a

serological survey of people diagnosed with CFS (as defined by the 1994 CFS case definition) was negative for antibodies to *M. fermentans* (Komaroff *et al.*, 1993). Our demonstration of the absence of *Mycoplasma* DNA by a sensitive amplification approach supports the negative serology study. Although cell-free plasma DNA may have limitations for monitoring the presence of *Mycoplasma* species, we believe this is unlikely, as numerous other cell-associated microbial pathogens can be detected readily in cell-free plasma DNA (Vernon *et al.*, 2002). As it has been shown that *Mycoplasma* species exist at relatively high rates in the peripheral blood of CFS patients in both the USA (Nasralla *et al.*, 1999) and Europe (Nijs *et al.*, 2002), it seems plausible that these microbial sequences should have been detected in plasma, reflecting death and lysis of infected cells.



**Fig. 1.** Optimized PCR for all four *Mycoplasma* species. Minimum amount of genomic DNA that yielded detectable amounts of PCR products was as follows: *M. fermentans*, 2.15 pg; *M. hominis*, 0.32 pg; *M. penetrans*, 2.72 pg; *M. pneumoniae*, 0.27 pg. Abbreviations: L, 100 bp DNA ladder; Mf, *M. fermentans*; Mh, *M. hominis*; Mp, *M. penetrans*; Mpn, *M. pneumoniae*.

Our goal was to reproduce the results of Nasralla *et al.* (1999) and Nijs *et al.* (2002) in a systematic way by using well-defined CFS subjects and non-fatigued controls. These authors detected the presence of four species of *Mycoplasma* DNA in patients with CFS and fibromyalgia. These studies used patients in tertiary care clinics rather than the general population and the results may reflect recruitment bias. Our study tested persons with CFS identified in the general population. Previous studies did not include appropriate controls, thus reported associations may be spurious. Our study enrolled non-fatigued controls from the same population as CFS cases. Finally, neither previous studies that reported an association nor our study, which shows no association, have been replicated independently. Independent reproducibility of PCR-based detection methods for infectious agents by other laboratories is an important factor for establishing an infectious cause of disease. Unfortunately, it is not uncommon to see variations in the rate of positivity in similar types of clinical sample from different laboratories that are engaged in PCR-based detection methods.

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