

## RNase L Levels in Peripheral Blood Mononuclear Cells: 37-Kilodalton/83-Kilodalton Isoform Ratio Is a Potential Test for Chronic Fatigue Syndrome

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**Chronic fatigue syndrome (CFS) is a disorder characterized by debilitating fatigue associated with immunological abnormalities. The etiology remains unclear. A low-molecular-mass (37 kDa) isoform of RNase L has been described in peripheral blood mononuclear cell (PBMC) extracts, and the ratio of two isoforms of RNase L (37 kDa/83 kDa) has been proposed as a potential biochemical marker of CFS. In a prospective case-control study, we tested whether the RNase L 37-kDa/83-kDa ratio could discriminate a SFC population. We compared the ratio of RNase L isoforms in PBMCs from 11 patients with CFS (6 women and 5 men; mean age  $\pm$  standard deviation, 43.2  $\pm$  13.8 years) and PBMCs from 14 healthy well-matched volunteers (10 women and 4 men; age, 39.1  $\pm$  11.6 years). A ratio of RNase L of 0.4 used as a threshold allowed diagnosis of CFS with high sensitivity (91%; 95% confidence interval [CI], 57 to 99%) and specificity (71%; 95% CI, 41 to 90%). The positive and negative prognostic values were 71% (95% CI, 41 to 90%) and 91% (95% CI, 57 to 99%), respectively. In the absence of acute infection or chronic inflammation, a high RNase L ratio could distinguish CFS patients from healthy volunteers. Additional large studies and follow-up studies are required to confirm the stability of this high ratio of RNase L isoforms in a CFS group.**

Chronic fatigue syndrome (CFS) is an illness characterized by long-lasting fatigue and other symptoms. Both psychological and biological etiological factors have been proposed, but the disorder remains of uncertain origin. No organism could be directly implied in the pathogenesis of CFS, and the physiopathology is still unclear. Because of the heterogeneous and nonspecific symptoms of CFS, a diagnostic test is needed.

Recently, CFS has been hypothesized to result from immune dysregulation. Particularly an up-regulation of key components of the 2',5'-oligoadenylate (2-5A)-RNase L pathway has been identified in extracts of peripheral blood mononuclear cells (PBMCs) from individuals with CFS (5, 6), including the presence of a low-molecular-mass form (37 kDa) of RNase L, whose origin remains undetermined. Two groups suggested that CFS occurs in an immune dysregulation background that would find its expression into a high ratio of RNase L isoforms, and one group did not.

In a prospective case-control study, we tested whether the 37-kDa/83-kDa ratio of RNase L isoforms could discriminate a SFC population.

### MATERIALS AND METHODS

**Patient characteristics.** The patient group consisted of 11 patients (6 women and 5 men; mean  $\pm$  standard deviation [SD] age: 43.2  $\pm$  13.8 years) fulfilling CFS criteria (2). The mean ( $\pm$ SD) duration of illness was 7.6  $\pm$  6.6 years. The control group consisted of 14 matched healthy volunteers (10 women and 4 men; mean  $\pm$  SD age, 39.1  $\pm$  11.6 years). A multidimensional fatigue inventory (MFI)

scale (4) was used to estimate fatigue in both groups. Before the inclusion, biological tests were performed for both groups, which allowed exclusion of organic diseases. Moreover, neither infectious history nor psychiatric illness was noted in the month prior to the start of the study. In the two groups, the mean ages were comparable, and the MFI score (maximal fatigue score = 100) was significantly higher in the patients (53.3  $\pm$  9.4) than in healthy volunteers (6.1  $\pm$  7). The clinical features of the patients are summarized in Table 1. This study was approved by the local ethics committee of the Hôpital Saint Antoine, Paris, France.

**PBMC preparation.** Venous blood samples were drawn in heparinized tubes. The samples were stored, and PBMCs were isolated within 4 h by density-gradient centrifugation. Blood (diluted 1/1 in phosphate-buffered saline) was layered onto 1 volume of Ficoll (density, 1.080; Gibco BRL, Paisley, Scotland) and centrifuged at 500  $\times$  g for 30 min at 20°C. PBMCs at the interface were collected and washed with 5 volumes of phosphate-buffered saline (500  $\times$  g, 15 min, 20°C). PBMC pellets were resuspended in 5 ml of erythrocyte-lysing buffer (155 mM NH<sub>4</sub>Cl, 10 mM NaHCO<sub>3</sub> [pH 7.4], 0.1 mM EDTA), centrifuged (500  $\times$  g, 10 min, 20°C), and frozen at  $-80^{\circ}\text{C}$  until use.

**Radiocovalent affinity labeling and analysis of 2-5A binding proteins.** A sensitive assay allowing the specific identification of 2-5A binding proteins in biological samples without a fractionating step was used. PBMC extracts were prepared in the presence of protease inhibitors to avoid artifacts from differential proteolysis.

A 3'-oxidized 2-5A [<sup>32</sup>P]pCp (3,000 Ci/mmol, 20,000 cpm) probe was incu-

TABLE 1. Clinical features of patients in this study<sup>a</sup>

| Group (n)            | Ratio of women to men | Age (yr)        | Fatigue duration (yr) | No. of CSF criteria fulfilled | MFI score      |
|----------------------|-----------------------|-----------------|-----------------------|-------------------------------|----------------|
| SCF (11)             | 6/5                   | 43.2 $\pm$ 13.8 | 7.6 $\pm$ 6.6         | 6.6 $\pm$ 1.3                 | 53.3 $\pm$ 9.4 |
| Controls (14)        | 10/4                  | 39.1 $\pm$ 11.6 | 0                     | 0                             | 6.1 $\pm$ 7    |
| P value <sup>b</sup> | 0.07                  | 0.48            | 0.001                 | 0.001                         | 0.001          |

<sup>a</sup> Values are means  $\pm$  SD unless otherwise noted.

<sup>b</sup> Mann-Whitney U test.

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TABLE 2. Diagnosis of CSF with an RNase L isoform ratio of 0.4 used as the cutoff

| RNase L isoform ratio<br>(37 kDa/83 kDa) | No. with diagnosis <sup>a</sup> |          |       |
|--|---------------------------------|----------|-------|
|  | CFS                             | Controls | Total |
| 0.4                                      | 10                              | 4        | 14    |
| 0.4                                      | 1                               | 10       | 11    |
| Total                                    | 11                              | 14       |       |

<sup>a</sup> Sensitivity, 91%; specificity, 71%; positive prognostic value, 71%; negative prognostic value, 91%.

bated with PBMC extracts (200 µg of protein) for 20 min at 4°C and for a further 20 min at room temperature with 20 mM sodium cyanoborohydride, allowing the covalent linkage of the probe to all 2-5A binding protein species. The extract was then fractionated by polyacrylamide gel electrophoresis (PAGE) with sodium dodecyl sulfate (SDS) as a denaturing agent. The size distribution of 2-5A-labeled polypeptides was visualized by autoradiography, and the relative abundance of the various bands was calculated by densitometric analysis with the NIH Image software. NIH Image is a public domain image processing and analysis program developed at the Research Services Branch of the National Institute of Mental Health.

**Statistical analysis.** Sensitivity, specificity, and positive and negative prognostic values were calculated with different threshold ratios of RNase L isoforms as a positive diagnosis, particularly ratios of 0.5, 0.4, and 0.3. Confidence intervals (95% CIs) were estimated. The ages, durations of fatigue, numbers of CFS criteria fulfilled, and MFI scores of the groups were compared by Mann-Whitney U test with significance at  $P < 0.05$ .

## RESULTS

SDS-PAGE fractionation of 2-5A-labeled polypeptides in PBMC extracts from healthy individuals gives rise to a major 83-kDa polypeptide band. An additional low-molecular-mass (37 kDa) 2-5A-labeled polypeptide can often be detected in extracts of CFS PBMCs, in keeping with previous reports (1).

The ratio of RNase L isoforms (37 kDa/83 kDa) of 0.4 used as a cutoff allowed discrimination of CFS patients from controls with high sensitivity (91%; 95% CI, 57 to 99%) and specificity (71%; 95% CI, 41 to 90%). The positive and negative prognostic values were 71% (95% CI, 57 to 99%) and 91% (95% CI, 41 to 90%), respectively. The results are summarized in Table 2. The sensitivity and specificity were lower when the other threshold values were used.

## DISCUSSION

Dysfunction of the 2-5A/RNase L antiviral pathway in patients with CFS has been reported by several groups (5, 6). In particular, a 37-kDa 2-5A binding polypeptide has been identified in 88% of CFS patients compared with only 28% of healthy control subjects (1).

The present study basically agrees with these data. These data were challenged by Gow et al. (3), who suggested that the high activity of the RNase L found in CFS patients was just a coincidence and probably reflected an ongoing viral infection. Indeed, reverse transcription-PCR analysis showed that RNase L mRNA levels did not differ between a CFS group and a healthy volunteer group. However, these data are not contradictory, since Gow et al. did not take into consideration the fact that the accumulation of 37-kDa isoforms of RNase L, as described here and as reported by De Meirleir et al. (1), may result from proteolysis of RNase L independently of RNase L mRNA level. In CFS, the accumulation of fragments with a molecular mass of 37 kDa could be due to an increased proteolytic activity in PBMC extracts.

In summary, apart from recent infection, our findings provide additional arguments to support the hypothesis that increased proteolytic activity leads to the accumulation of a 37-kDa binding polypeptide in PBMC extracts from CFS patients and could be considered as a potential diagnostic marker. In the absence of acute infection or chronic inflammation, a high RNase L isoform ratio could distinguish CFS patients from healthy volunteers. Additional large studies and follow-up studies are required to confirm the stability of this high ratio of RNase L isoform in a CFS group.

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