

# Multiple sclerosis-associated retrovirus (MSRV) in Sardinian MS patients

**Abstract**—Blood and CSF of Sardinian patients with MS and neurologic control subjects were tested for MS-associated retrovirus (MSRV). CSF detection in MS was 50% at clinical onset, increasing with temporal disease progression, and 40% in control subjects. In blood, MSRV was detected in all MS patients, in most patients with inflammatory neurologic diseases, and rarely in healthy blood donors. MSRV may represent a marker of neurologic diseases of inflammatory origin.

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The complex, multifactorial autoimmune cascade developing in MS lesion formation is sustained by genetic and environmental susceptibility factors, including various infectious agents.<sup>1</sup> Many intracellular pathogens are implicated in MS etiology, although none of them have withstood the test of time.<sup>2</sup> An MS-associated retrovirus (MSRV)<sup>3</sup> has been linked to MS pathogenesis, and its possible role is under study. MSRV belongs to the human endogenous retrovirus-W family<sup>4</sup> and produces extracellular virions, found in plasma and CSF of MS patients, having reverse transcriptase activity.<sup>3,5</sup> To analyze its possible MS-specific role, we searched for MSRV in CSF and blood of patients with active MS and with other neurologic diseases (OND). Blood MSRV circulation among the general population also was analyzed.

**Patients and methods.** A cohort of 113 individuals of ascertained Sardinian origin was studied; subjects were free of immunomodulatory treatments (azathioprine, interferon- $\beta$ , cyclophosphamide, or steroids) for at least for 3 months before samples were taken from CSF or blood. Informed consent was obtained previously.

**Patients with MS.** Thirty-nine consecutive patients diagnosed with definite MS<sup>6</sup> were enrolled (female-to-male ratio, 1.8). The 24 relapsing-remitting (RR) patients, 4 secondary-progressive (SP) patients with known MS (6 months or longer), and those at MS onset (11 patients) were in the active phase of disease (development within 2 weeks of new neurologic signs attributable to demyelination). Diagnosis of patients at onset subsequently was confirmed based on evidence of clinical or paraclinical dissemination of neurologic signs from MS<sup>6</sup>: seven had clinically probable MS (four women, three men), and four had laboratory-supported definite MS (women). The re-

maining 28 patients had clinically definite MS (17 women, 11 men; mean MS duration,  $7.3 \pm 5.8$  years).

**Neurologic control subjects.** Thirty-five patients with OND were diagnosed as having infectious and postinfectious encephalomyelitis (4), immune-mediated peripheral neuropathy (3), epilepsy (5), neurosis (4), ischemic stroke (3), compressive radiculomyelopathy (3), migraine (3), trigeminal neuralgia (2), myasthenia gravis (2), CNS vasculitis (1), thyrotoxicosis (1), ALS (1), head trauma (1), hereditary degenerative peripheral neuropathy (1), and recurrent optic neuritis of unknown origin (1, monolateral in the absence of detectable MRI lesions and CSF abnormalities).

**Healthy control subjects.** Thirty-nine matched healthy blood donors (BD) at the Blood Transfusion Center of Sassari Health District were used as control subjects of MSRV blood circulation in the overall population.

Cell-free plasma and CSF samples were filtered through 0.45- $\mu$ m membranes (Millex, Millipore Co, MA), precipitated with polyethylene glycol<sub>8000</sub> for 24 hours, centrifuged at  $5000 \times g$  for 20 minutes, and treated with bovine ribonuclease I A (USB Corporation, Cleveland, OH) to avoid contamination by cellular RNA containing endogenous retroviral sequences; RNA samples then were extracted and digested with RNase-free deoxyribonuclease I (Amersham Life Science, Buckinghamshire, UK) to avoid possible DNA contamination; RNA samples then were re-extracted. Only extracellular, encapsidated RNA, therefore, was available for molecular study.<sup>3,5</sup> Coded samples of virionic RNA were used as a template for nested reverse transcription (RT)-PCR, as described elsewhere,<sup>7</sup> using primers specific for the *MSRV-pol* gene.<sup>5</sup> Controls included PCR of RNA not exposed to RT with primers specific for the  $\beta$ -globin gene (Synthetic Genetics, San Diego, CA)<sup>7</sup> or with MSRV-specific primers (to ensure the absence of contaminating cellular and endogenous retroviral DNA sequences), PCR of cellular DNA samples without template (negative control), and samples of human cell DNA (positive control).

Statistical analysis was performed by using the Epi Info2000 Database and Statistics Software Program (CDC/WHO, Atlanta, GA). Two-tailed Fisher's exact test, Mann-Whitney *U* test, and  $\chi^2$  calculation were used as required.

**Results.** The CSF samples were available for 31 MS and 10 OND patients. Extracellular virus could be detected in 80.6% MS and 40% OND CSF samples ( $p = 0.04$ , table 1). MSRV-positive OND were affected by CNS vasculitis (1), trigeminal neuralgia (1), ALS (1), and recurrent optic neuritis of unknown origin (1).

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**Table 1** Presence of MSRV in the CSF of patients with MS and patients with OND with or without neurologic inflammatory diseases

Group	No. of patients	Age, y, mean $\pm$ SD (range)	% MSRV+	p Value*
Patients with MS	31	36.7 $\pm$ 10.4 (19–59)	80.6	
Patients with OND (all)	10	31.9 $\pm$ 9.0 (22–48)	40.0	0.04
OND (CNS inflammatory disease)	4	35.0 $\pm$ 7.0 (25–40)	50.0	NS†
OND (non-CNS inflammatory disease)	6	29.9 $\pm$ 10.1 (22–48)	33.3	0.03

\* Two-tailed Fisher's exact test, *p* value vs patients with MS.

† The difference between OND with and without inflammatory diseases is not significant.

MSRV = MS-associated retrovirus; NS = not significant; OND = other neurologic disease.

Patients with MS were then stratified according to disease duration, diagnostic level,<sup>6</sup> clinical course, and CSF oligoclonal band number. Virus positivity was detected in 50% of CSF samples at clinical onset (figure, A and C) and increased according to disease duration (*p* = 0.009; see the figure, A), to diagnostic level (*p* = 0.003; see the figure, B), and to MS course (from onset to SP forms; *p* = 0.01; see the figure, C). MSRV positivity already was 78% in CFS

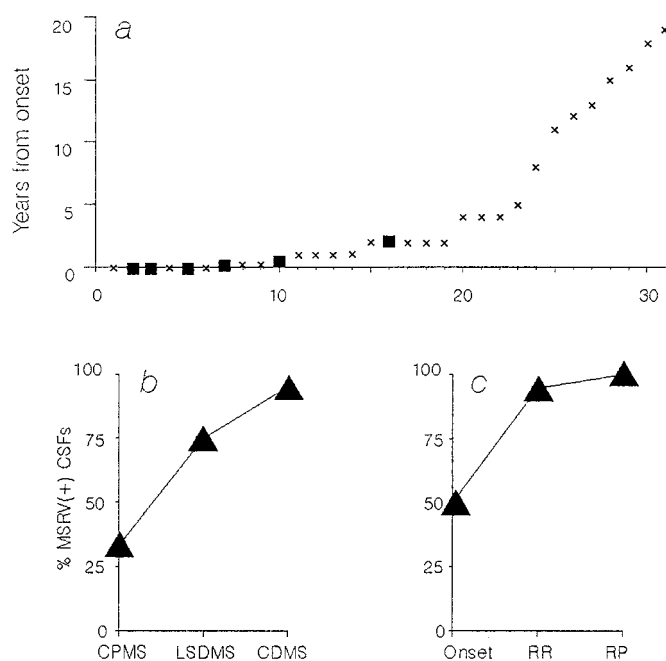
samples of subjects presenting with three OCB and 100% in those having more than six OCB, without significant difference (not shown).

Plasma MSRV (table 2) was detected in 12.8% of healthy BD and in 100% of patients with MS (*p* < 0.000001); 42.9% of OND patients also were MSRV positive (*p* = 0.004 vs BD, and *p* < 0.000001 vs MS). MSRV positivity was 63.6% in the 11 OND patients with inflammatory disease (4/5 patients with inflammatory CNS diseases, 3/4 with inflammatory peripheral neuropathy, 0/2 with MG) and in 33.0% of the remaining subjects with OND (ischemic stroke, 2; epilepsy, 1; thyrotoxicosis, 1; neurosis, 1; ALS, 1; migraine, 1; and trigeminal neuralgia, 1). Notably, MSRV positivity of OND patients with inflammatory CNS disease and peripheral neuropathy was 77.8% (*p* = 0.0003 vs BD, and *p* = 0.03 vs MS).

**Discussion.** Sardinia is a high-risk area for MS: its prevalence (144 cases per 100,000 inhabitants) and incidence (7 new cases per 100,000 inhabitants/year) rates are among the highest worldwide<sup>8</sup>; moreover, MS incidence increased in the last four decades by almost threefold. Because a 40-year span is too short a time interval for a substantial change in Sardinians' genetic pool to occur, an explanatory environmental change might be assumed. Notably, small population-based epidemiologic studies suggest that in central Sardinia, MS started suddenly, after a possible exposure to novel environmental factors.<sup>8</sup> We therefore studied MSRV circulation among Sardinian MS patients and healthy individuals to search for a possible correlation between high MS prevalence and MSRV presence in this population.

We found extracellular MSRV in 50% of MS patients' CSF at clinical onset and a correlation with disease duration. Although direct correlation with MS progression cannot be proven unless repeated lumbar punctures are performed on the same individual, we observed that CSF MSRV positivity has an incremental trend that is related to overall disease progression (from onset to secondary progression), being present in 100% of untreated, active SP MS patients. Conversely, 40% of CSF samples from subjects with OND tested positive (*p* = 0.04 vs MS).

The complete (100%) concordance between MS and plasma MSRV, as opposed to 12.8% positivity of



**Figure.** Extracellular MS-associated retrovirus (MSRV) in CSF from patients with MS according to parameters related to temporal disease progression (a): CSF MSRV positivity according to disease duration (y axis, years from disease onset). Each point represents an individual, either virus negative (■) or virus positive (x). (a) MSRV in the CSF of patients with MS (mean values for MSRV– and MSRV+ patients: 0.4  $\pm$  0.8 and 5.6  $\pm$  6.3 years, respectively; Mann–Whitney U test, *p* = 0.009). (b) Percent MSRV positivity according to Poser's diagnostic criteria<sup>6</sup> ( $\chi^2$  calculation, *p* = 0.003). (c) Percent virus positivity with respect to the form of the disease, from onset to relapsing-remitting (RR) to secondary-progressing (SP) forms ( $\chi^2$  calculation, *p* = 0.01). CPMS = clinically probable MS; LSDMS = laboratory-supported definite MS; CDMS = clinically defined MS.

**Table 2** Presence of MSRV in the blood of patients with MS and patients with OND with or without inflammatory diseases in comparison to blood donors

Group	No. of patients	Age, y, mean $\pm$ SD (range)	MSRV, <sup>†</sup> %	<i>p</i> Value*	
				vs BD	vs MS
Blood donors	39	37.0 $\pm$ 10 (19–59)	12.8		
Patients with MS	39	36.9 $\pm$ 9.4 (19–59)	100	<0.000001	
Patients with OND (all)	35	36.5 $\pm$ 11.5 (19–57)	42.9	0.004	<0.000001
OND (inflammatory disease)	11	36.0 $\pm$ 10.0 (23–57)	63.6	0.002	0.001
OND (non-inflammatory disease)	24	36.8 $\pm$ 12.3 (19–57)	33.0	ns <sup>†</sup>	<0.000001

\* Two-tailed Fisher's exact test.

<sup>†</sup> Pearson's  $\chi^2$  *p* = 0.05.

BD = blood donors; MSRV = MS-associated retrovirus; ns = not significant; OND = other neurologic disease.

healthy controls, is impressive. Also, 42.8% of OND plasma samples were MSRV positive, differing from both patients with BD (*p* = 0.004) and patients with MS (*p* < 0.000001). When results from OND subjects were stratified with respect to inflammatory diseases (see table 2), MSRV presence in blood appeared to be related to the inflammatory nature of the diseases, particularly those of both peripheral and CNS origin, with a significant difference from MS patients as well as from BD; the difference between the remaining OND subjects and BD, however, was not significant.

Detection of MSRV in MS does not create a new causative agent of MS. Rather, it reflects properties of viruses that could explain the pathogenesis of complex diseases in an interactive way. We also found MSRV in a few patients with BD and in 40% of patients with OND. Moreover, MSRV has been found in synovial fluids of rheumatoid arthritis patients,<sup>9</sup> therefore excluding an MS-restricted presence. However, many commonly circulating viruses may be activated under unspecified circumstances, and only in given conditions do they develop their full pathogenic potential. Similarly, MSRV might be endogenously produced or may circulate safely in humans, and in the presence of predisposing conditions, it could contribute to MS reactivation.

Even if MSRV merely represents an epiphenomenon, its detection in body fluids of patients deserves further study to elucidate pathogenic connections (i.e., gliotoxicity)<sup>10</sup> aimed at possible future therapeutic strategies.

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