Presence of Infectious XMRV in Blood Cells of Chronic Fatigue Syndrome (CFS) Patients

CFS:CDC Criteria (Fakuda,1994)

- Persistent or relapsing fatigue of 6 months or longer in duration, generally has a distinct onset
- Other known medical conditions excluded by clinical diagnosis
- Patients have at least 4 of the following symptoms:

✓ Impaired memory or concentration
✓ Sore throat
✓ Tender cervical or axillary lymph nodes
✓ Muscle pain

✓ Multi-joint pain
✓ New headaches
✓ Un-refreshing sleep
✓ Post exertional malaise lasting more than 24 hours

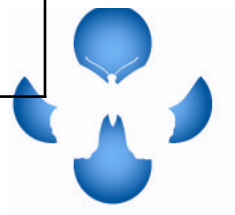
Nevada CFS Cohort:

- Between 1984 and 1987, a cluster of 300 cases of CFS was identified in Incline Village, Nevada. For these studies of immunological and viral parameters:
- RNA, DNA, plasma and frozen PBMC from ~100 of this cohort collected at two time points: Sept 06 and July 07.
- RNA, DNA, plasma from 320 normal regional controls collected between 2004-2008

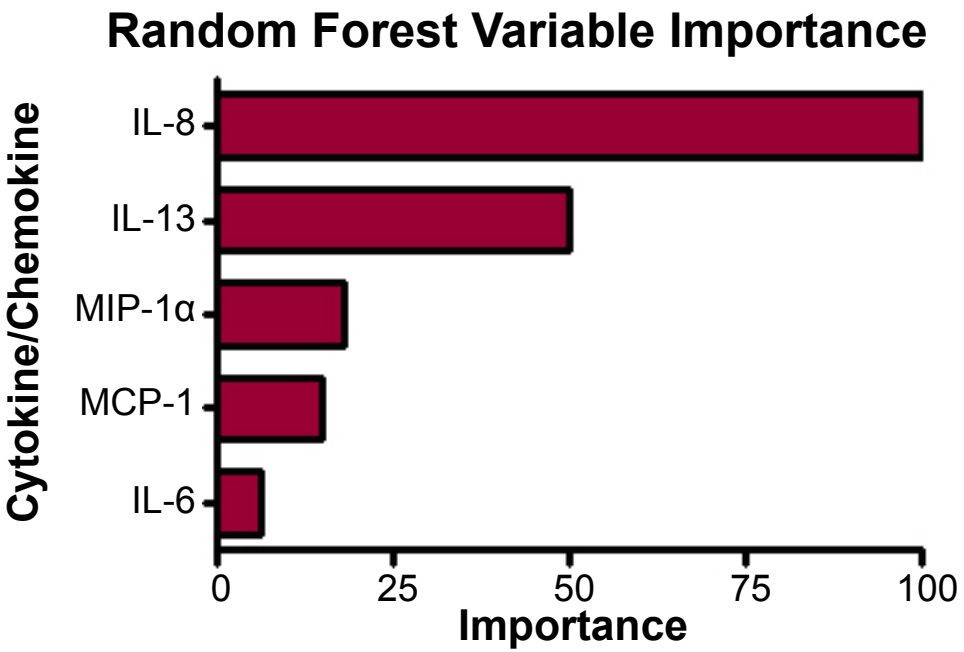
Inflammatory Cytokines/Chemokines are Dysregulated in CFS

CYTOKINES/ CHEMOKINES	Patient N = 118	Control N=138	P value	FUNCTION IN INFLAMMATION
IL-8	1045	13	<0.0001	RNase L and CMV activated
MIP-1α	763	91	0.0062	Elevated in Neurodegenerative disease
MIP-1β	1985	164	<0.0001	Elevated in Neurodegenerative disease
IL-6	336	29	<0.0001	Stimulates chronic inflammation
TNF-α	148	13	<0.0001	Stimulates chronic inflammation
IL1β	500	56	<0.0001	Stimulates chronic inflammation
IP-10	98	32	<0.0001	Interferon response protein
IFN-α	35	60	<0.0001	Stimulates macrophages and NK cells to elicit an anti-viral response
IL-13	28	86	<0.0001	Inhibits inflammatory cytokine production
IL-7	160	60	<0.0001	Stimulates proliferation of B and T lymphocytes and NK cells

Mean values in pg/ml: Red denotes up regulation,
Blue denotes down regulation



Random Forest Predicts 5 Cytokine/Chemokine Signature of CFS with 94% Specificity and Sensitivity



Random Forests Prediction Success

Actual Class	Total Cases	Percent Correct	Control N=135	Patient N=121
Control	138	93.48	129	9
Patient	118	94.92	6	112

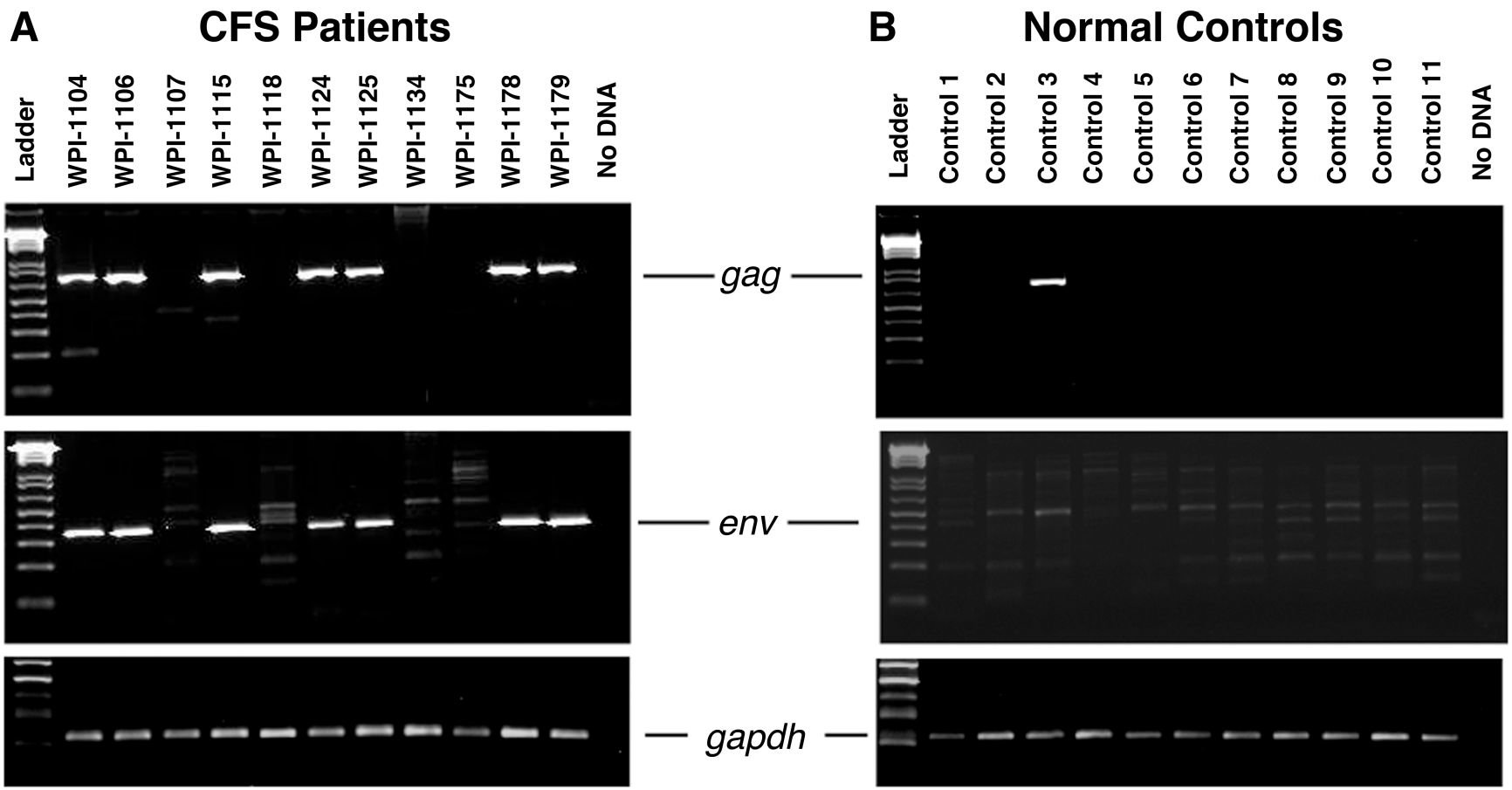
Rationale:

CFS is a multi-system disorder manifested by inflammatory sequelae including:

- antiviral enzyme (RNase L) dysfunction
- low natural killer (NK) cell numbers and function
- innate immune activation

Could these patients be infected with XMRV?

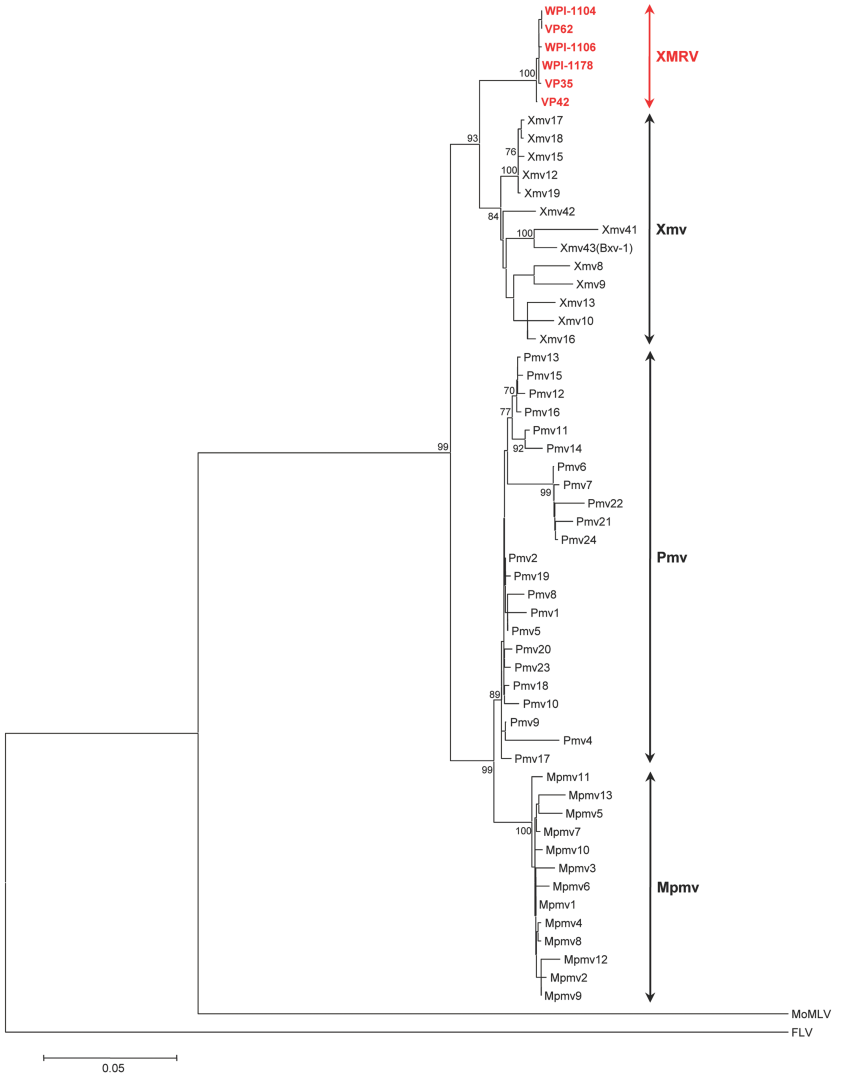
Presence of XMRV Sequences in Human DNA

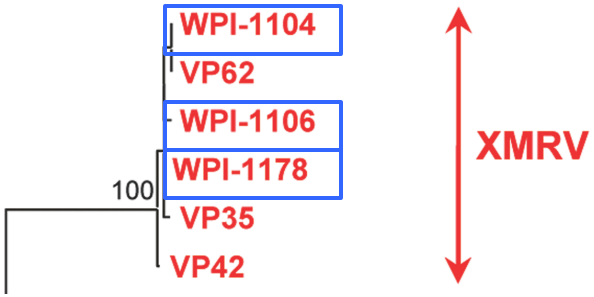


68/101 (67%)

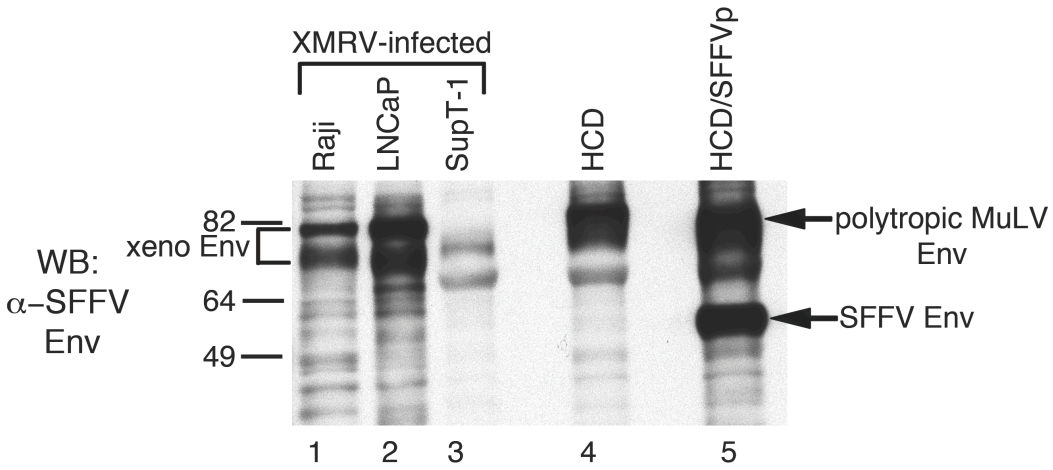
12 /320 (3.75%)

Phylogenetic analysis revealed that XMRV isolates from prostate cancer and CFS form a distinct branch within non ecotropic MLVs

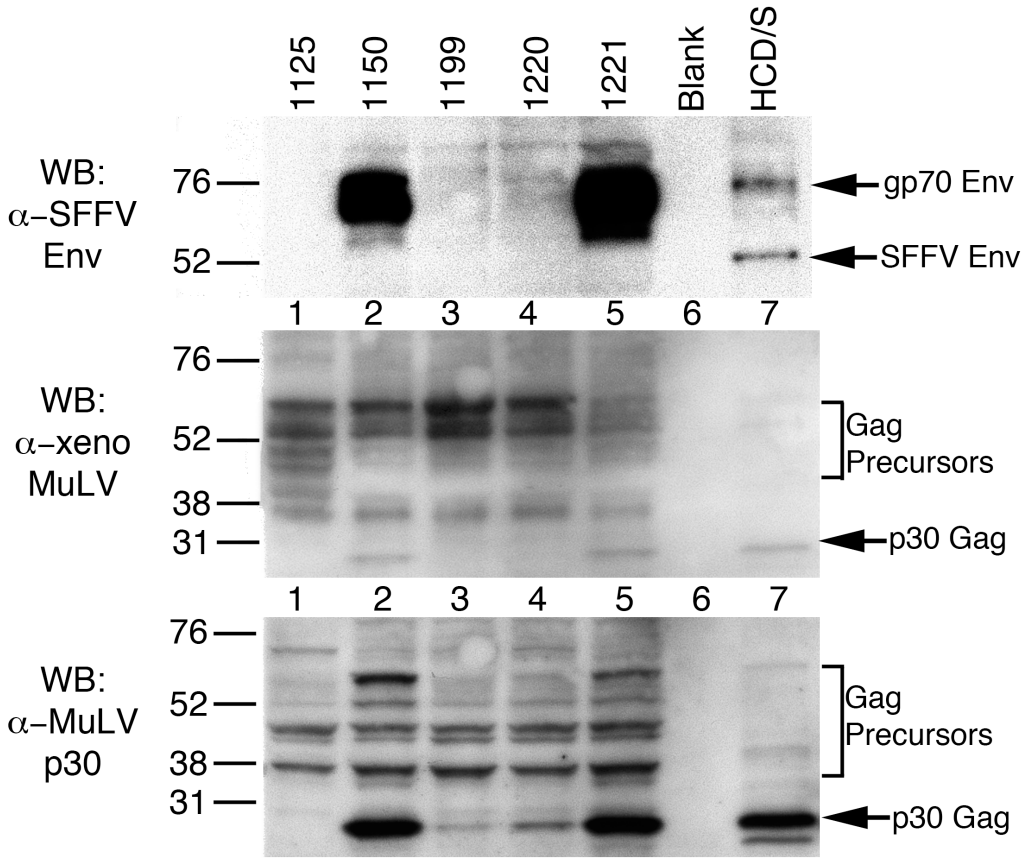




Detection of XMRV Env in Human Cells Using a Monoclonal Antibody to SFFV Env

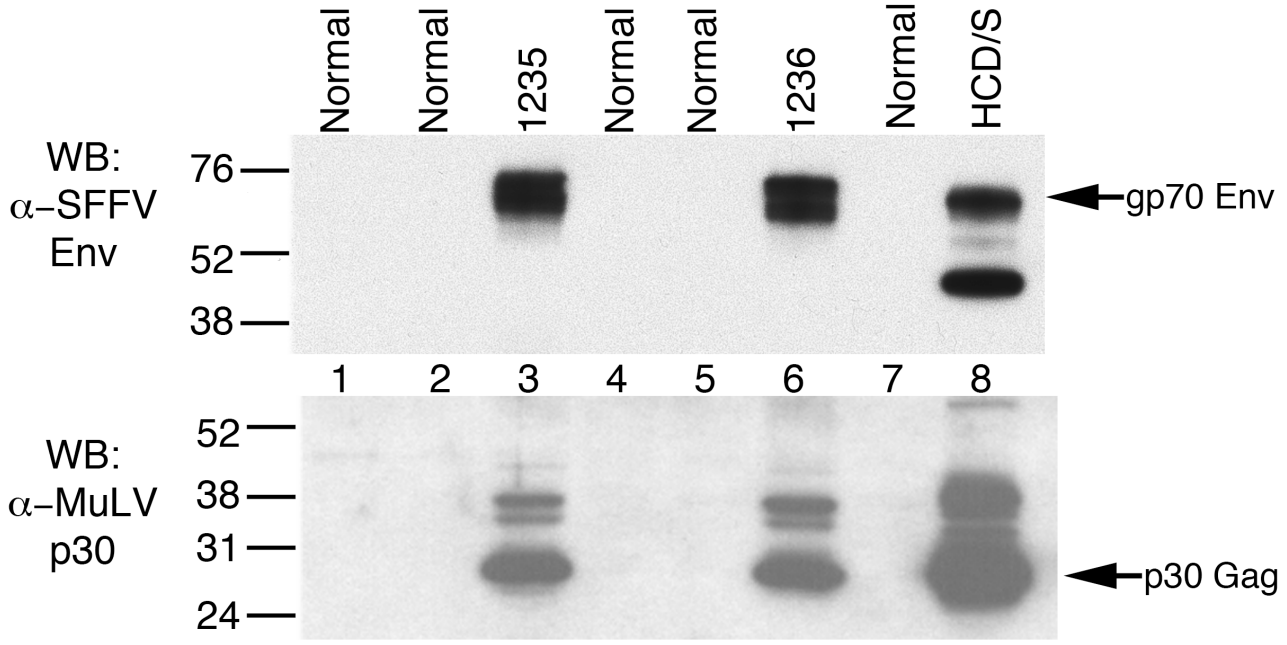


Detection of XMRV Protein Expression in Activated PBMC from CFS Patients



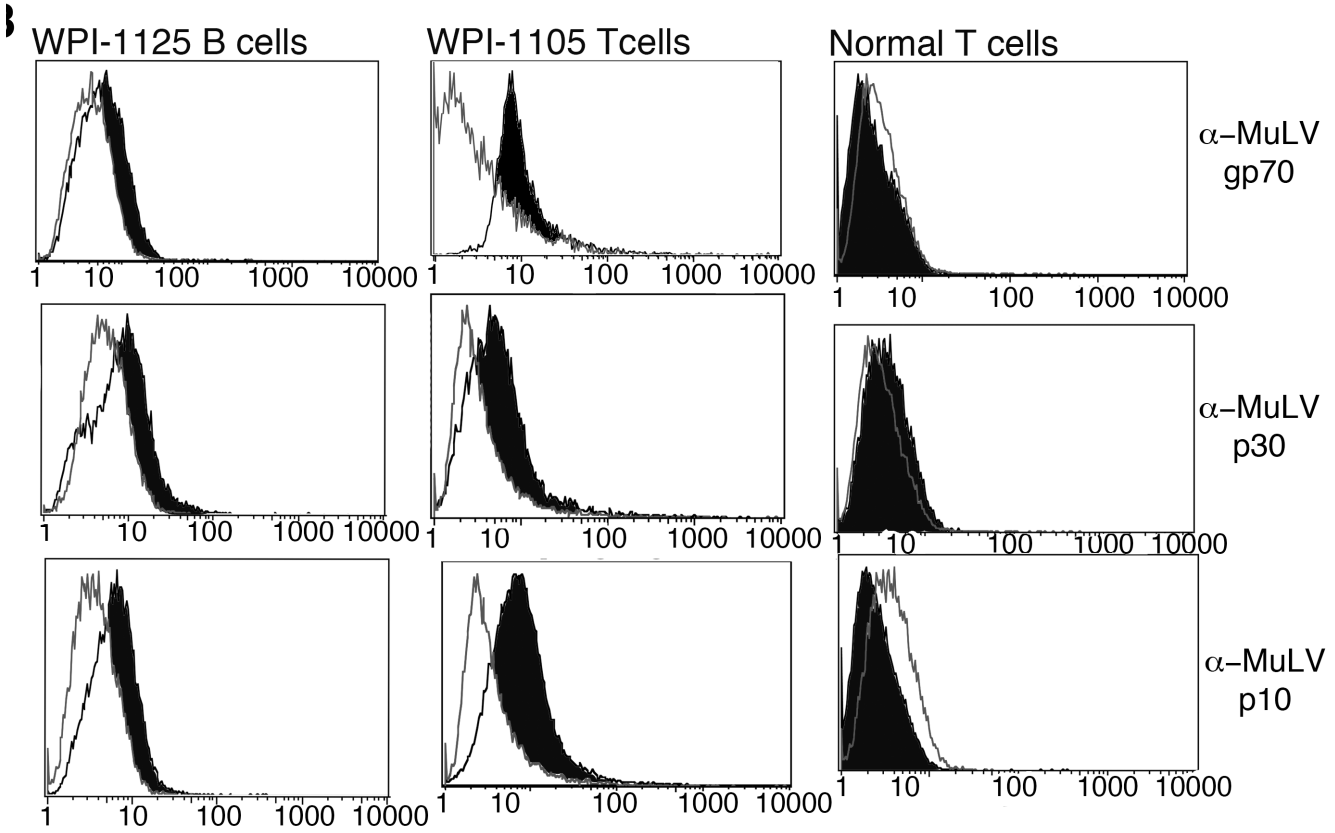
34 out of 50 (68%)

Absence of XMRV Protein Expression In Activated PBMC from Normal Donors

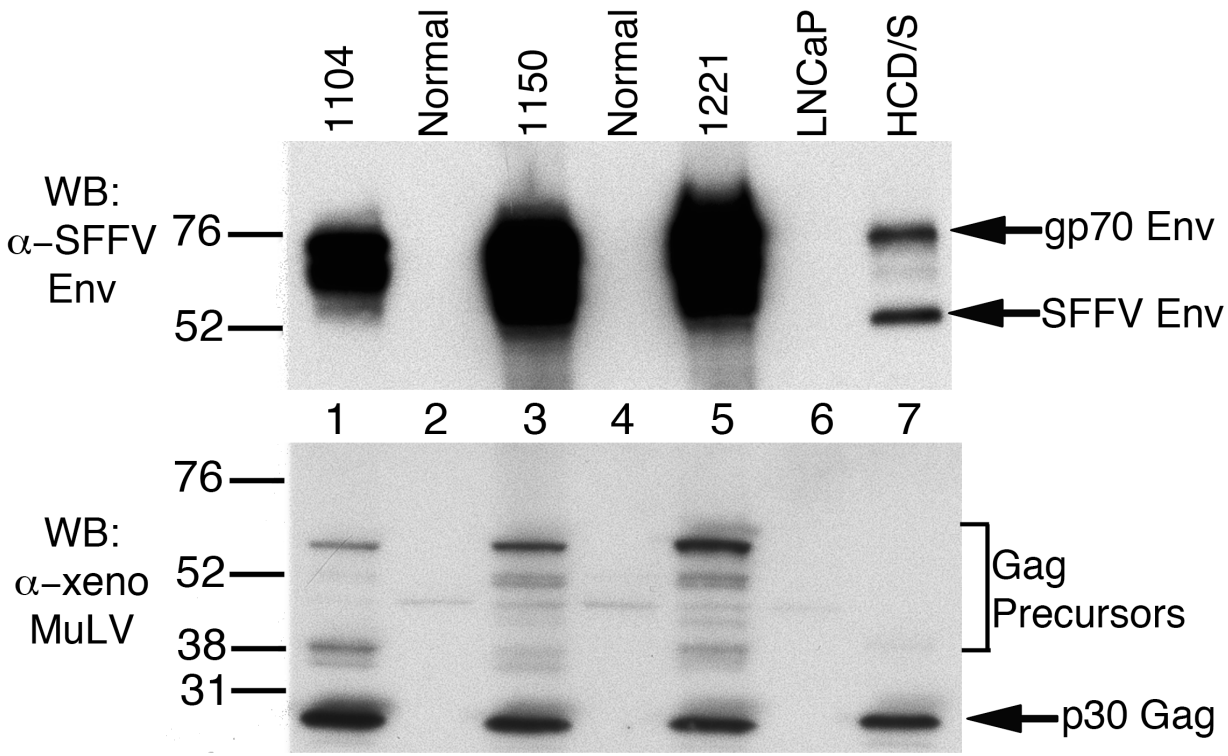


24 Normal Donors from NIH Clinical Center

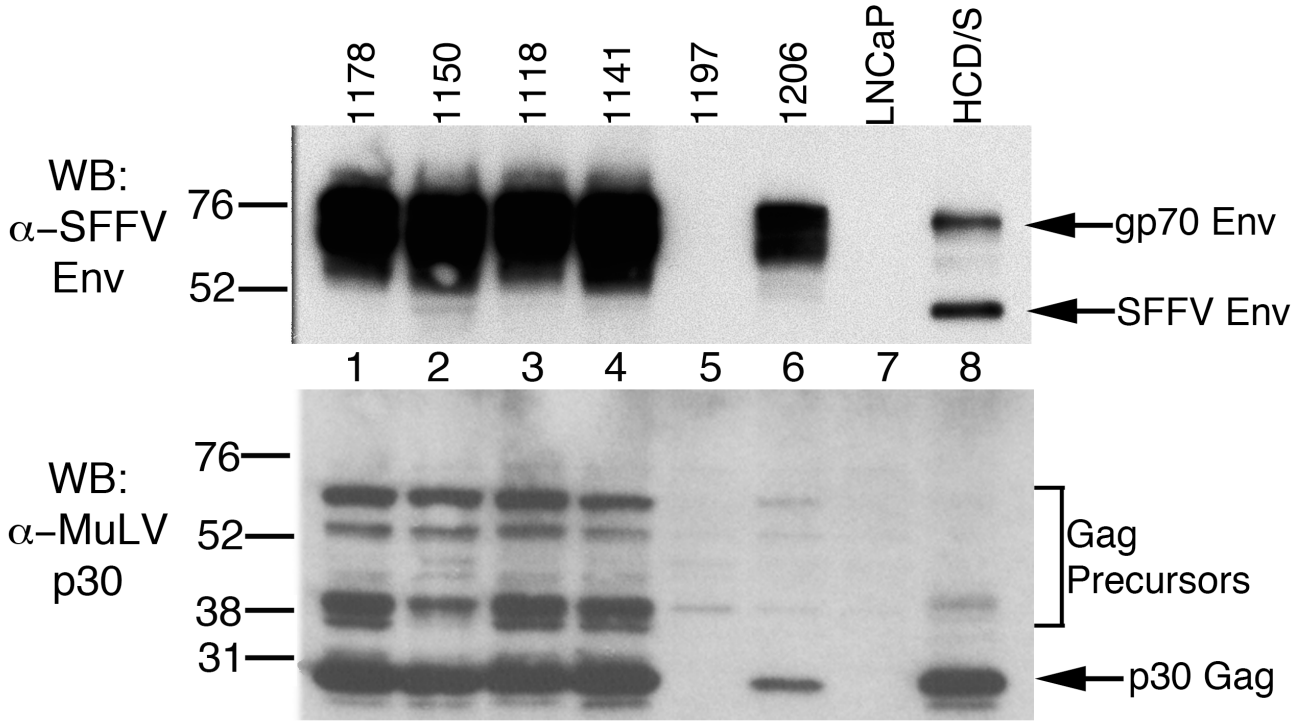
XMRV Protein Expression in Purified Activated T and B Lymphocytes from CFS Patients



Transmission of XMRV from Activated PBMC to LNCaP

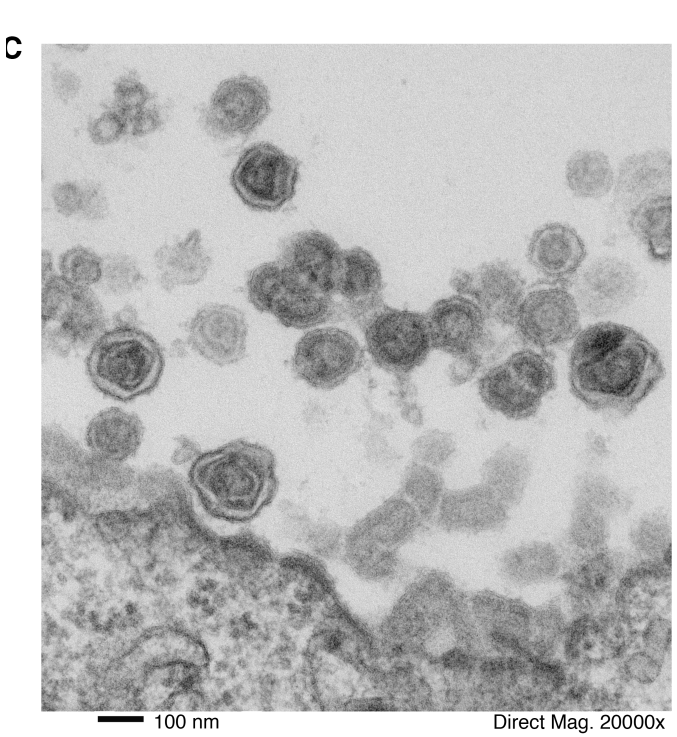


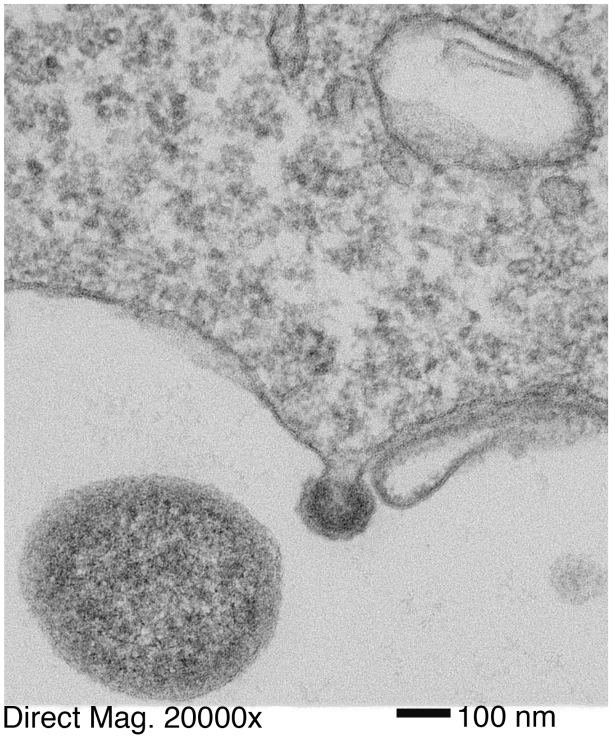
Transmission of XMRV from CFS Patients' Plasma to LNCaP



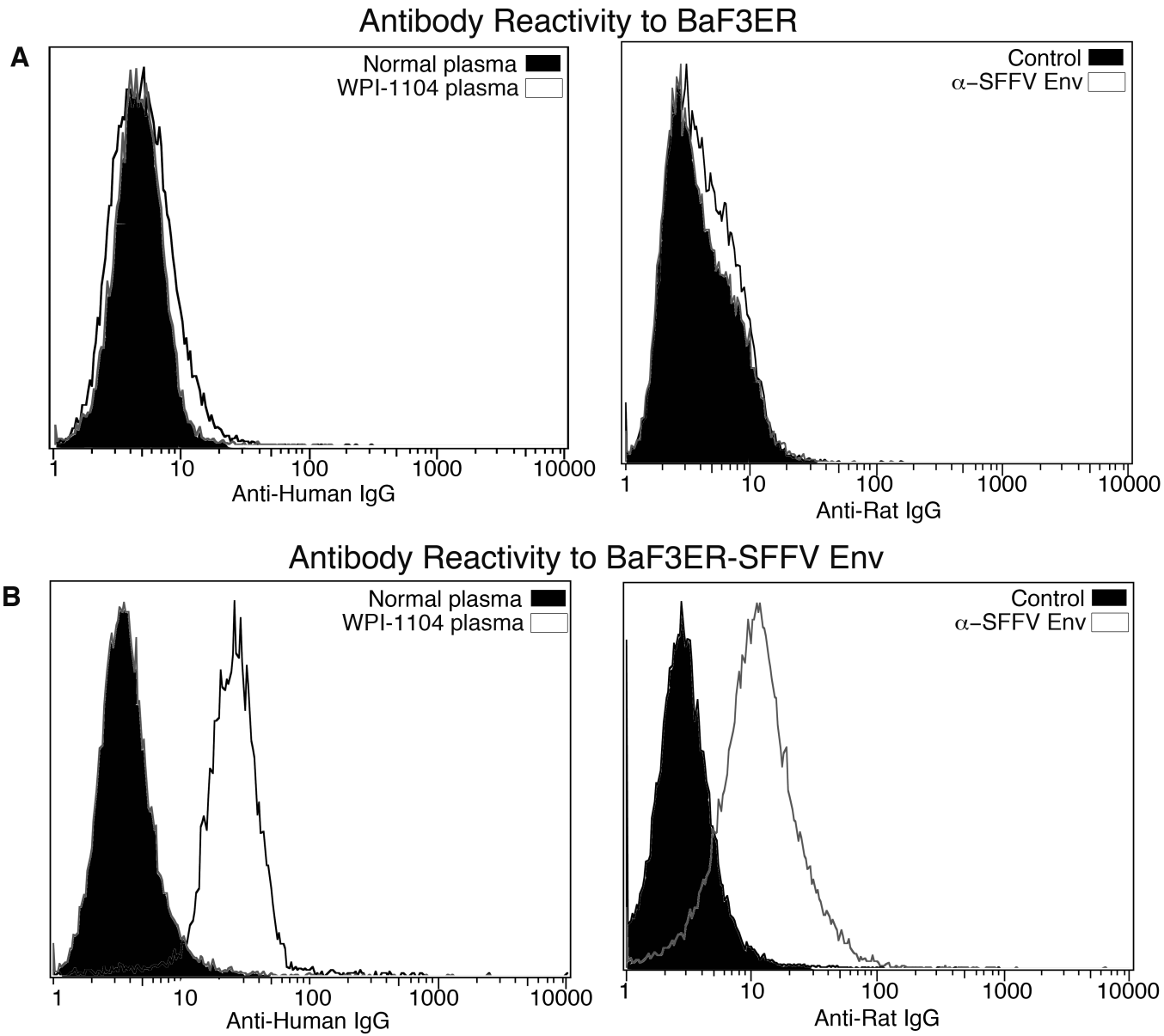
21 positive of 25 (84%)

Transmission Electron Micrograph of C-type Retrovirus Particles Transmitted from CFS Plasma to LNCaP

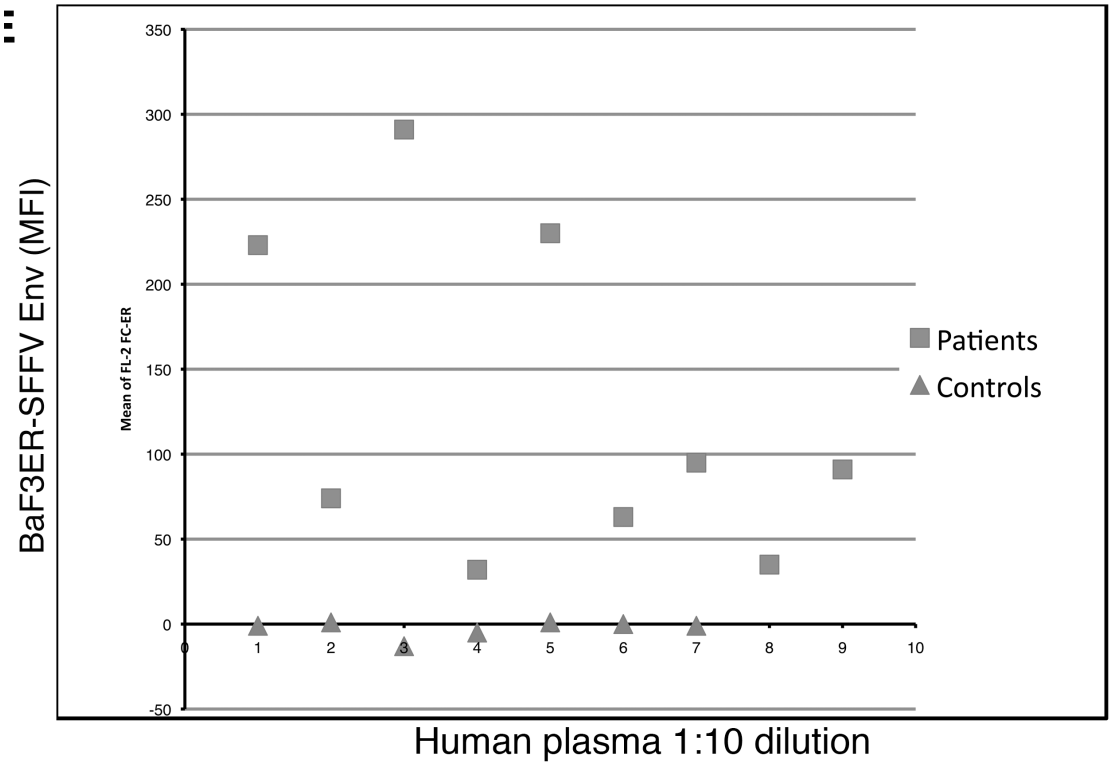




Antibodies in CFS Patients' Plasma to XMRV Env



Detection of Antibodies to XMRV Env in CFS Patient Plasma



XMRV Infection in CFS Patients Does Not Correlate with the RNASEL R462Q Variant

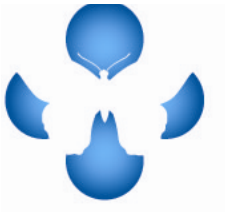
Genotype	*Population*		*XMRV Results*	
R462Q variant	**Patients**	**Controls**	**Negative**	**Positive**
AA	16	13	3	4
AG	66	36	18	26
GG	74	39	27	33
Total	**156**	**88**	**48**	**63**
X^2	0.10	0.87	0.11	
P value	0.95	0.65	0.74	

XMRV Expression in Carolina/Florida Cohort

- 9/15 (60%) positive for XMRV gag DNA from fresh PBMC
- 13/15 (86.7%) positive by western for XMRV Env and Gag upon co-culture of plasma or PBMC with LNCaP
- 8/15 (53%) plasma samples contain antibody to XMRV Env

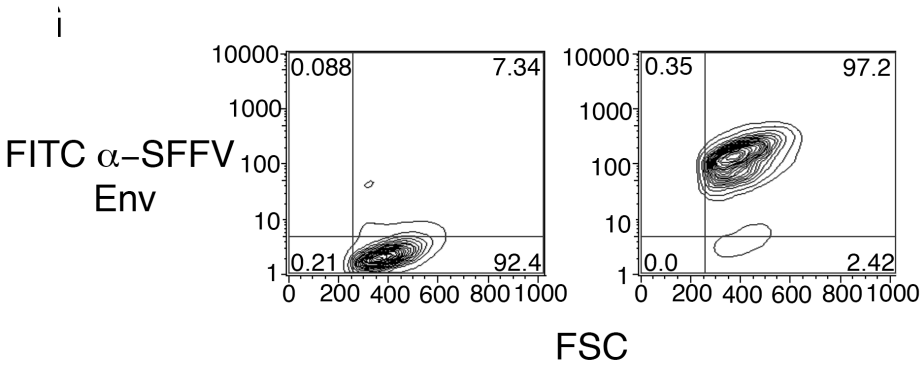
Nevada CFS Patients with Cancer

ID#	XMRV status	Clonal TCRγ	Lymphoma/cancer
987	positive	positive	MCL
1028	positive	negative	Thymoma
1185	positive	negative	myelodysplasia
1282	positive	Positive + IGH	MCL
2119	positive	positive	Lymphoma
2740	positive	positive	Previous Lymphoma
1674	positive	positive	Lymphoma
1068	Not tested	Not tested	Thymoma
2166	Not tested	Not tested	MCL
1928	positive	positive	Thymoma
2814	positive	Not tested	lymphoma
1849	positive	positive	MCL
1467	positive	positive	suspicious
2776	positive	Positive + IGH	suspicious
1127	positive	positive	CLL
2833	Not tested	Not tested	MCL
1987	positive	Not tested	CLL
2151	positive	positive	CLL
2152	positive	positive	MCL
2157	positive	positive	suspicious



WPI-1282 CFS Diagnosis One Decade Prior to MCL

- 1988---Seen at NIH for CFS
- 1998---Splenectomy to decrease aggressiveness.
- 2000---Seen at NIH for mantle cell lymphoma. Given Rituxan and Velcade
- 2004---BMT with adult stem cells
- 2008---Blast crisis MCL … death



Conclusions:

- Infectious XMRV found in lymphocytes & plasma from >75% of CFS patients
- XMRV in CFS and Prostate Cancer are closely related
- form distinct phylogenetic branch
- An immune response to the virus was detected in some CFS patients
- XMRV creates in CFS patients a neuro-immune deficiency predisposing to cancer
- Data suggest that the human population is at risk from infection of XMRV

Challenges:

- Diagnosis
- Therapy
- Vaccine

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