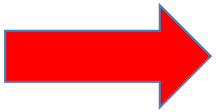
Biomarker in CFS/ME
Current status

- Many studies on altered biomarkers
- Most alterations only in subsets of CFS/ME patients/ overlap with controls
- most studies were performed in single centers using
- non standardized assays and various case definitions
- Assays based on flow cytometric cell phenotyping or functional assays analysing cytokine production or cytotoxic function are difficult to standardize



No diagnostic biomarker available yet

Immune biomarker in CFS

Biomarkers	References
>IL-10, IFNγ, TNFα by PHA stimulated lymphocytes; >CD4+CD25+ T cells expressing FoxP3 and VPACR2; <cytotoxic activity of NK and CD8+T cells; <granzyme and >perforin by gene expression in CFS/ME compared to HC at rest.	Brenu, et al., 2011
>IL-4, IL-5, IL-12, LTα, IL-1α, IL-1β, IL-6; <IL-8, IL-13, IL-15; ≥IL-2, IFNγ, IL-17, IL-23, TNFα in plasma of CFS/ME compared to HC at rest.	Fletcher, et al., 2009
Cytokine co-expression networks distinct in CFS/ME compared to HC. Subjects at rest.	Broderick, et al., 2010
<Perforin in NK cells and CD8+T cells by quantitative flow cytometry. CFS/ME compared to HC at rest	Maher, et al., 2005
<Perforin by gene expression in GWI compared to HC at VO_2Max in exercise challenge	Whistler, et al, 2009
<NK cell cytotoxicity; < plasma dipeptidyl peptidase IV;>T-cell activation. CFS/ME compared to HC at rest	Fletcher, et al., 2010a
In CFS/ME compared to HC: absence of significant increase in IL-6 & TNFα following exercise challenge	Jammes, et al., 2009
IL-1β, IL-12, IL-6, IL-8, IL-10, and IL-13 elevated at 8 hrs post exercise in subjects showing symptom flair at 48 hrs.	White, et al, 2010
>NPY in CFS/ME subjects compared to HC at rest;	Fletcher, et al., 2010b
no exercise related change for NPY, IL-6, IL-10, IL12, TNFα in CFS/ME but > in HC	Harvey, et al., 2011
<Serum vitamin E, a marker for oxidative stress in CFS/ME compared to HC at rest	Miwa & Fujita, 2010
Exercise related <plasma F(2)-isoprostanes (marker of oxidative stress); No effect of exercise on plasma IL-6 or sIL-6R in CFS/ME or HC.	Robinson, et al., 2010
In most CFS/ME but not in HC, exercise >transcription for most sensory and adrenergic receptors and one cytokine which correlated with fatigue and pain.	Light, et al, 2012
Metabolic syndrome predictors elevated in CFS/ME compared to HC at rest.	Maloney, et al, 2010
Increased lactate levels in ventricular cerebrospinal fluid of CFS/ME compared to HC at rest.	Murrough et al. 2010
Significant deficiencies in mitochondrial function in CFS/ME compared to HC at rest.	Myhill et al., 2009
Quantitative proteomics using high resolution mass spectrometry of CSF, unique patterns associated with CFS compared to HC and Lyme disease at rest.	Schutzer, et al., 2011
Unique CFS/ME spinal fluid proteome of 60 proteins when compared to HC and GWI. The CFS/ME and GWI patients shared 20 unique proteins at rest.	Baraniuk et al 2005
> CRP, >8-iso-prostaglandin F(2 alpha) isoprostanes in CFS/ME compared to HC at rest	Spence, et al, 2008
<LPS induced pro-inflammatory cytokines under psychological stress in CFS/ME compared to HC at rest	Gaab et al, 2005
> CRP in CF cases not meeting the CFS/ME definition; no difference between CFS/ME and HC at rest	Raison et al, 2009
Abnormal pattern of cortisol over 24 hours associated with elevated fatigue.	Torres-Harding, et al 2009
<Cortisol levels and flattened diurnal release of cortisol) associated with a poorer response to CBT in CFS/ME.	Roberts, et al., 2010
Variations in the 5' region of NR3C1 (glucocorticoid receptor gene) in CFS/ME compared to HC at rest.	Rajeevan et al., 2007
HPA axis dysfunction in CFS/ME compared to HC at rest.	Papadopoulos & Cleare, 2011
HPA axis dysfunction CFS/ME compared to HC at rest.	Ben-Zvi, et al., 2009
No evidence for biomarkers using gene expression in a twin study.	Byrnes et al., 2009
Significant evidence for a heritable contribution to predisposition to CFS/ME.	Albright, et al, 2011
Gene expression revealed 'CFS signature genes'.	Kerr, et al., 2008
Reassessment of 'CFS signature genes' failed to confirm predictive ability.	Frampton, et al., 2011

Brain Behav Immun. 2012 Nov;26(8):1202-10. doi: 10.1016/j.bbi.2012.06.006. Epub 2012 Jun 23.

Biomarkers for chronic fatigue.

Klimas NG[1], Broderick G, Fletcher MA.

EUROMENE WG on biomarkers

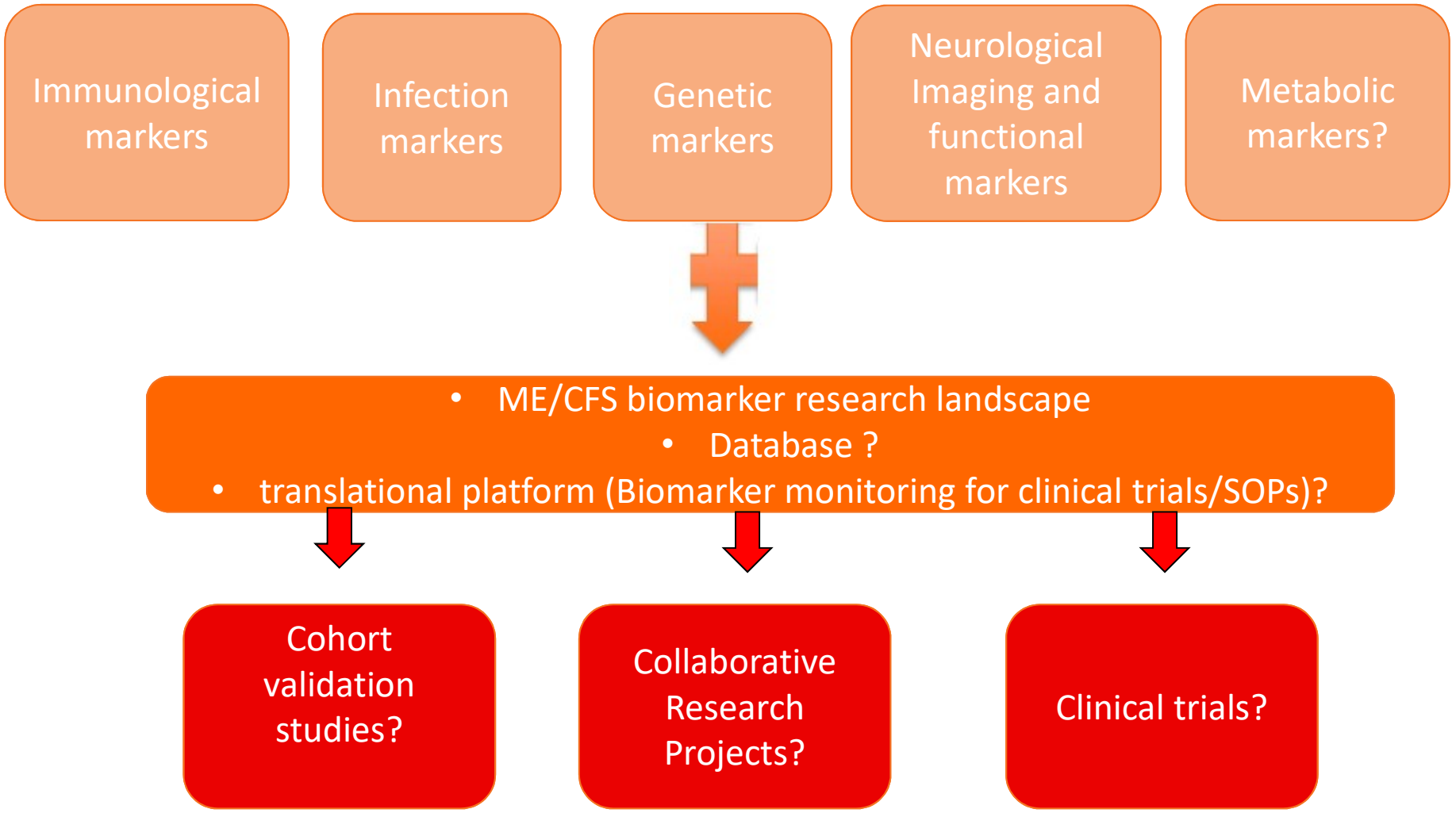
The objectives:

1) Establish special interest groups within the network able to take fragmented research in a harmonised way

2) Survey in EU countries existing data
on potential biomarkers in ME/CFS:

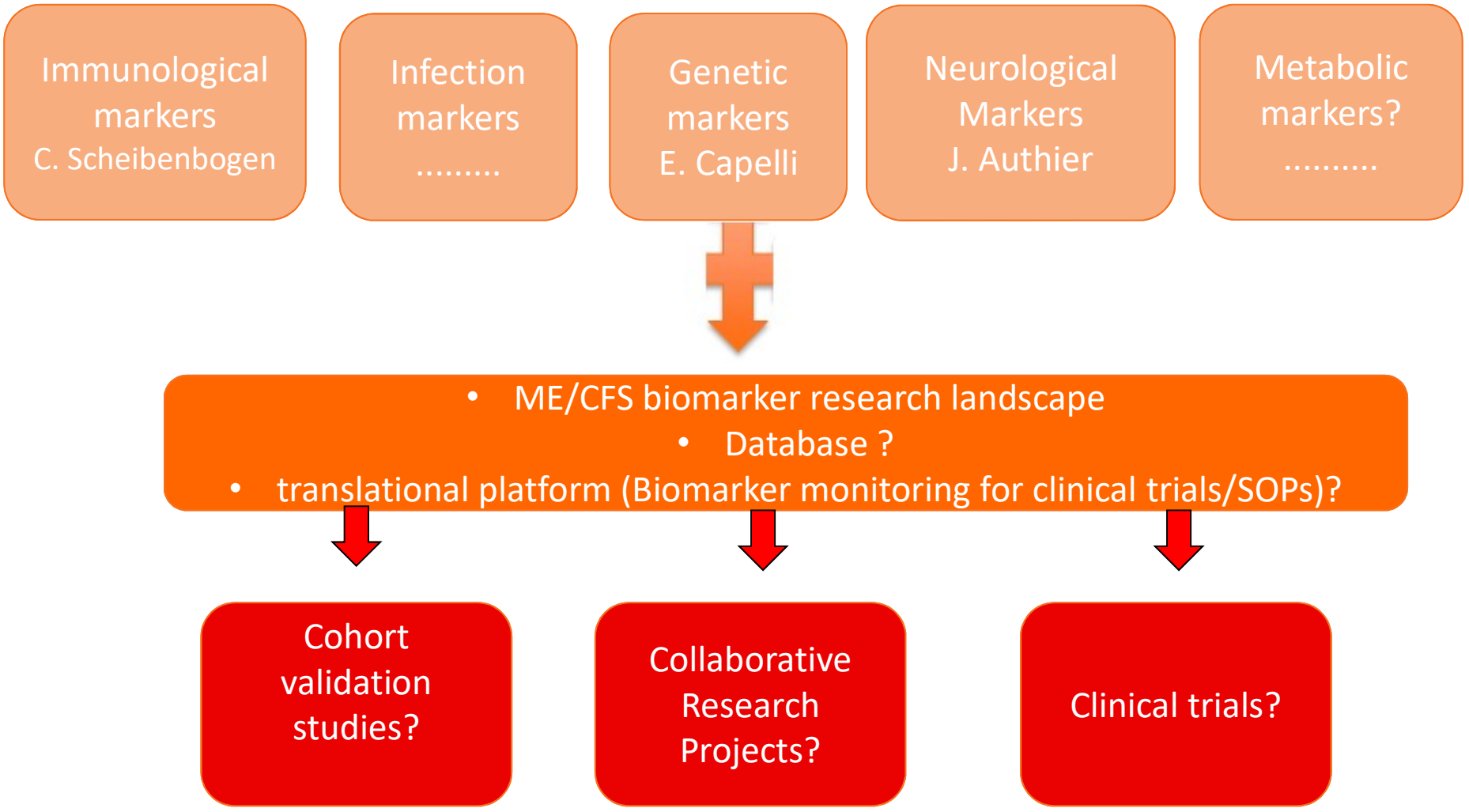
- immunological
- infection-associated
- genetic and epigenetic biomarkers,
- neuro-imaging/neuro markers

EUROMENE WG on biomarkers

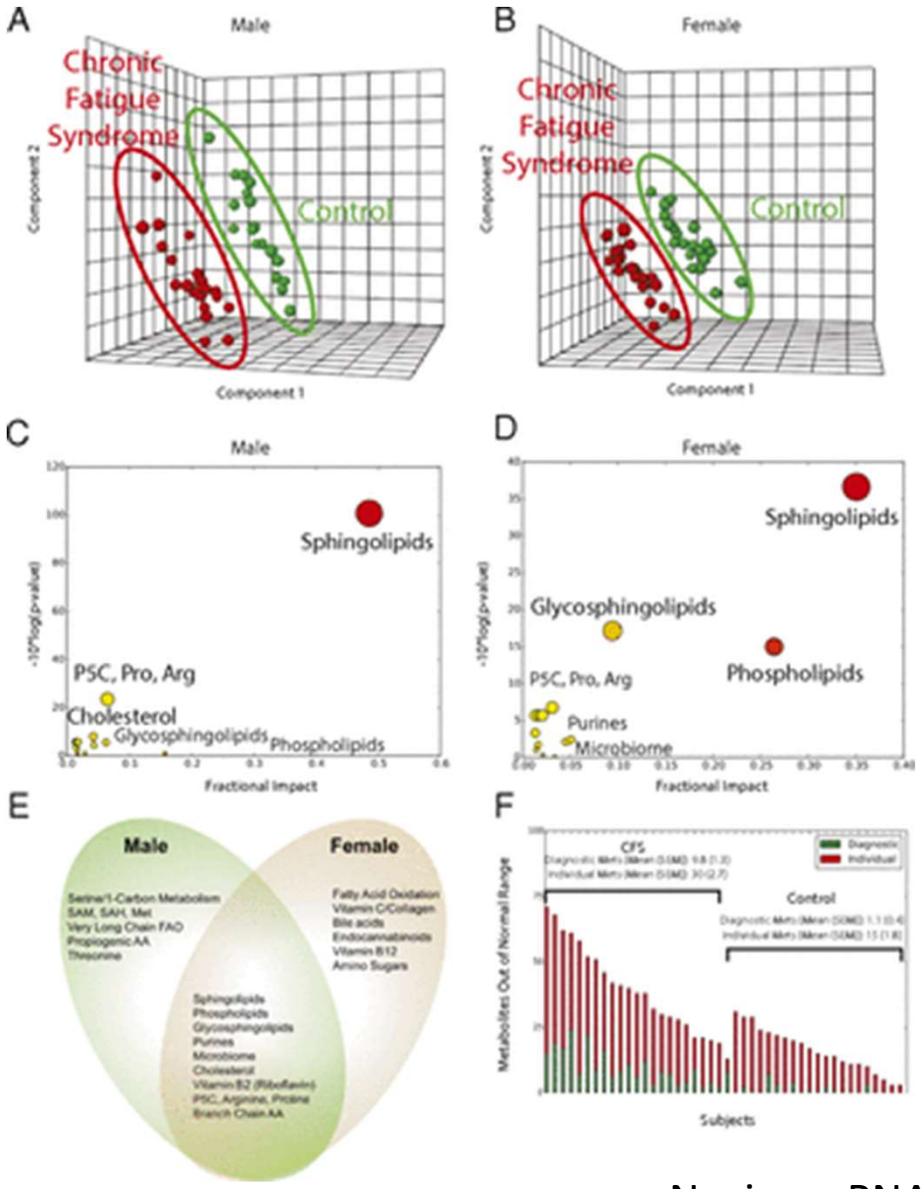


COST is supported by the EU Framework Programme Horizon 2020

EUROMENE WG on biomarkers



COST is supported by the EU Framework Programme Horizon 2020



Naviaux, PNAS 2016

EUROMENE WG on biomarkers

Immunological markers C. Scheibenbogen	Infection markers	Genetic markers E. Capelli	Neurological Markers J. Authier	Metabolic markers? .....

How to achieve our goals?

2) Survey on biomarker to establish an „**European biomarker landscape**“

- biomarker/research groups/fundings?
  - Organize via MC members for each country?
  - via Pub med survey?

1) Establish special interest groups within the network able to take **fragmented research** in a harmonised way.
   - Review?

Reviews on biomarker in CFS

Jason LA, **Zinn** ML, **Zinn** MA.
Myalgic Encephalomyelitis: Symptoms and Biomarkers
Curr Neuropharmacol. 2015:701-34. Review.

Blundell S, Ray KK, Buckland M, White PD.
Chronic fatigue syndrome and circulating cytokines: A **systematic** review.
Brain Behav Immun. 2015 Nov;50:186-95.

Fischer DB, William AH, Strauss AC, Unger ER, Jason L, Marshall GD Jr, Dimitrakoff JD. Chronic Fatigue Syndrome: The Current Status and Future Potentials of Emerging **Biomarkers**.
Fatigue. 2014 Jun 1;2(2):93-109.

Klimas NG, Broderick G, Fletcher MA.
Biomarkers for chronic fatigue.
Brain Behav Immun. 2012 Nov;26(8):1202-10.

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   - Review?
   - Critically appraised topics e.g. autoantibodies, soluble marker, NGS, lipids?

Critical appraised topics

1. **Ask**ing a focused question
2. **Search**ing for the best available evidence
3. Critically **Apprais**ing the evidence for validity and clinical relevance
4. **Apply**ing the results to clinical practice
5. **Evaluation** of performance

1. **Ask**: Are there soluble markers which may be suited as diagnostic markers in CFS?
2. **Search** Single studies, Reviews, Expert reviews
3. **Appraise** Critical reading of studies
4. **Apply** Performing selected assays in single cohorts
5. **Evaluation** in various labs and different cohorts

sCD26 as biomarker

PLoS One. 2010 May 25;5(5):e10817. doi: 10.1371/journal.pone.0010817.

Biomarkers in chronic fatigue syndrome: evaluation of natural killer cell function and dipeptidyl peptidase IV/CD26.

Fletcher MA[1], Zeng XR, Maher K, Levis S, Hurwitz B, Antoni M, Broderick G, Klimas NG.

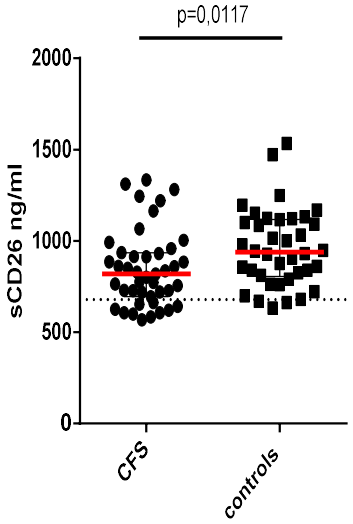
Variable	*Number of CFS Cases*	*Median (25–75th percentile)*	*Number of Healthy Controls*	*Median (25–75th percentile)*	*p*
NKCC%	176	12 (8–21)	230	28 (20–37)	.000
% CD26+CD2+ Cells	75	61 (55–66)	100	52 (47–59)	.000
sCD26 in Serum (ng/ml)	73	489 (396–643)	122	671 (496–871)	.000
MoI CD26/CD2+ Cell	77	3625 (2844–4633)	102	4388 (3600–5388)	.001

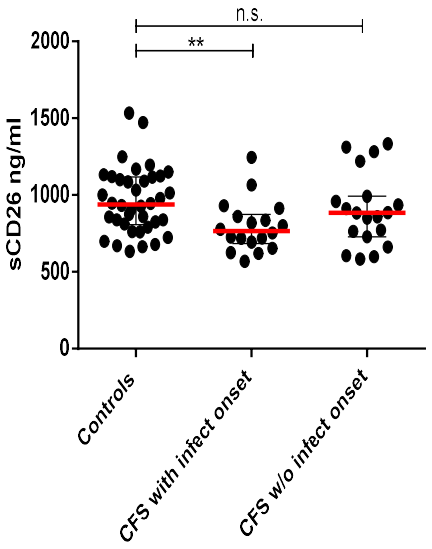
[a]**>80% female, average age 48;**
[b]**>80% female, average age 47.**
doi:10.1371/journal.pone.0010817.t001

confirmatory studies:

- Hanevik K, 2012
- Fenouillet 2016

Own data





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   - Review?
   - Critically appraised topics e.g. autoantibodies, soluble marker, NGS, lipids?
   - Validate in EUROMENE network

EUROMENE WG on biomarkers

Immunological markers
C. Scheibenbogen

Infection markers
.....

Genetic markers
E. Capelli

Neurological Markers
J. Authier

Metabolic markers?
.....

- ME/CFS biomarker database and research landscape
- translational platform (Biomarker monitoring for clinical trials/SOPs)

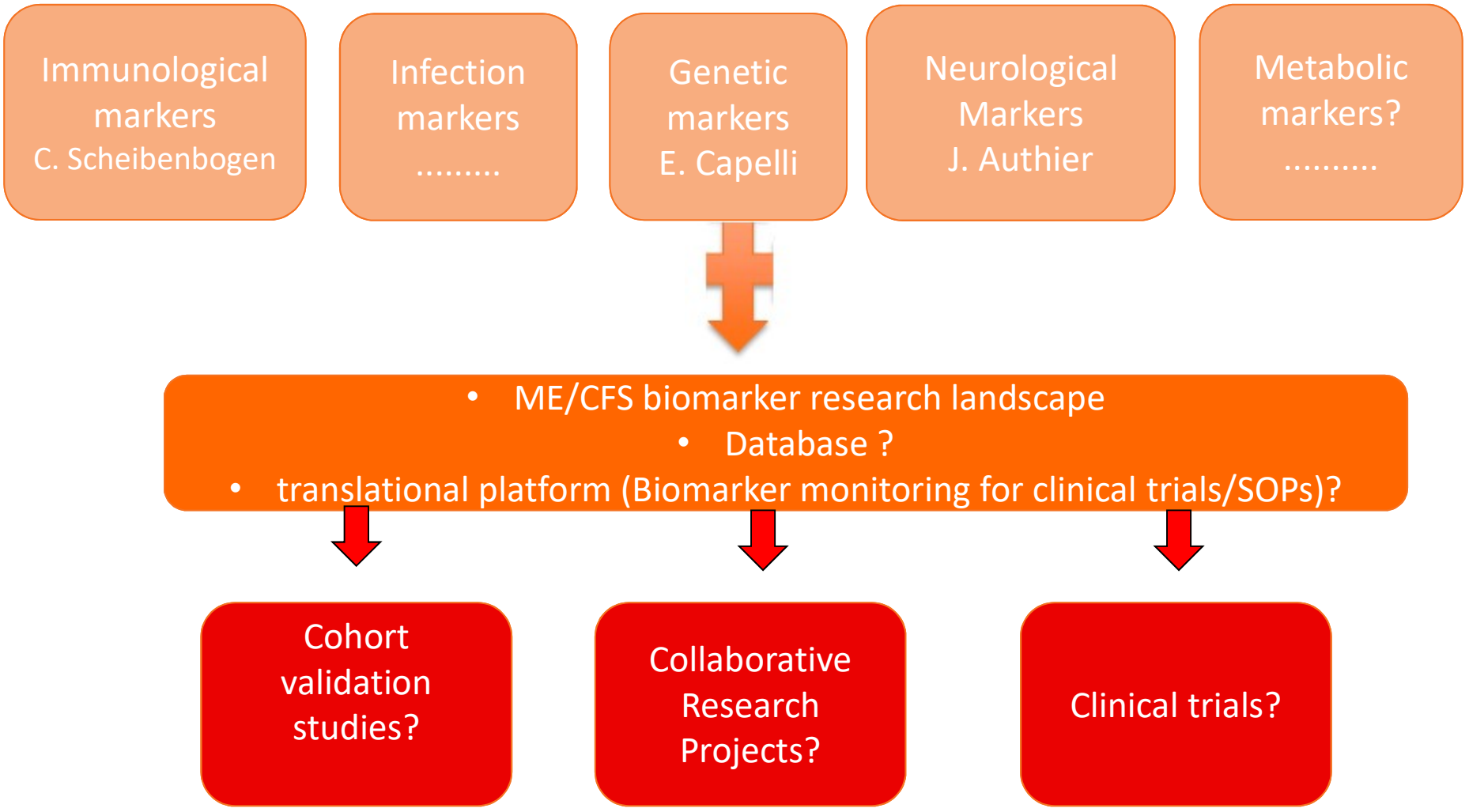
Cohort validation studies

How to achieve this?

- Cohort/Longitudinal studies of selected markers?
- Correlation with clinical data?


cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

EUROMENE WG on biomarkers



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EUROMENE WG on biomarkers

Immunological biomarkers

- Autoantibodies
  C. Scheibenbogen
- Genetics
  E. Capelli
- CSF J. Blomberg?
- Exercise response?
- .....
- .....
- .....
- .....

Neurological Imaging and functional Markers
J. Authier

Metabolic biomarkers
J. Blomberg?

Infection biomarkers
.....

Autontibodies in CFS/ME

Navaneetharaja et al. **A Role for the Intestinal Microbiota and Virome in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)?** J. Clin. Med. 2016

Table 3. Autoantibodies in ME/CFS patients.

Antigen Target of Autoantibodies	Reference
Cardiolipin	Hokama *et al.*, 2008 [99] Hokama *et al.*, 2009 [100]
Nuclear envelope antigens	Konstantinov *et al.*, 1996 [101]
Neuronal cell	Buchwald *et al.*, 1991 [102]
68/48 kD protein antibodies	Nishikai, M., 2007 [103]
Serotonin, microtubule-associated protein 2 and muscarinic cholinergic receptor-1	Bassi *et al.*, 2008 [104]
5-HT, gangliosides and phospholipids	Klein and Berg, 1995 [105]
Muscarinic cholinergic receptor	Tanaka *et al.*, 2003 [97]

EUROMENE WG on biomarkers

Immunological biomarker

Infection biomarker

Genetic biomarker

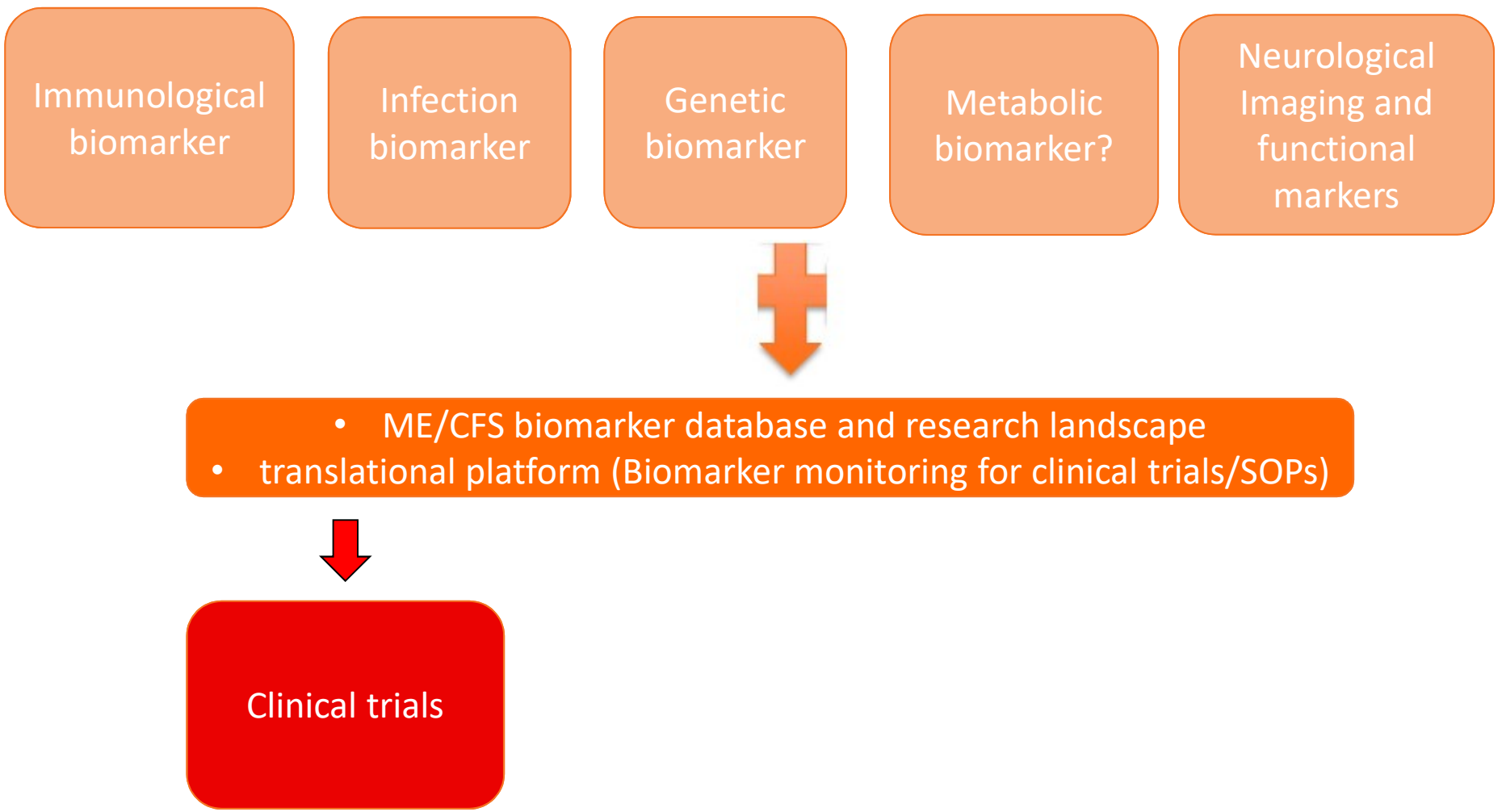
Metabolic biomarker?

Neurological Imaging and functional markers

- ME/CFS biomarker database and research landscape
- translational platform (Biomarker monitoring for clinical trials/SOPs)

Collaborative Research Projects

EUROMENE WG on biomarkers



Autoantibodies against neurotransmitter receptors in CFS/ME

Collaboration with Dr. Heidecke, Fa. Celltrend, Luckenwalde
and Drs. Fluge/Mella, Universität Bergen

Cohorts:

286 Berlin CFS/ME patients, 25 Bergen CFS/ME patients
108 healthy controls

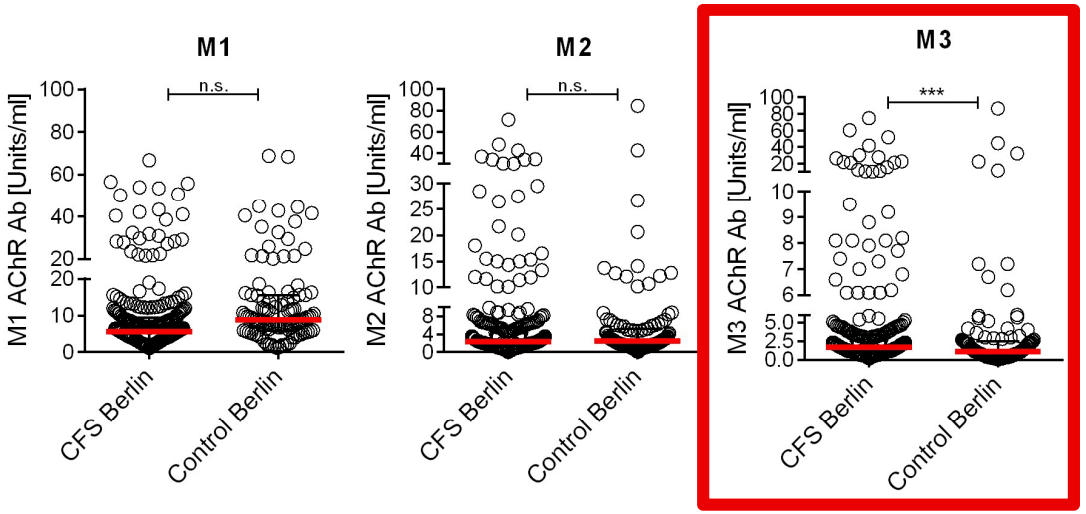
ELISA analysis of serum autoantibodies against:

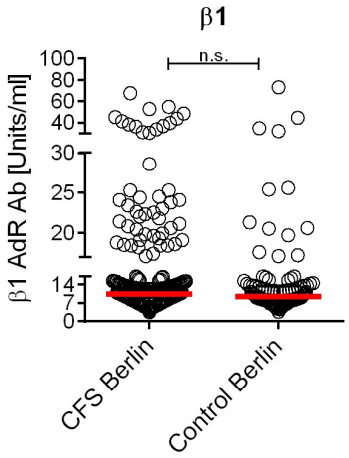
- Muscarinergic acetylcholine receptor (M1-5)
- Adrenergic receptor (ß1+2, α1)
- Dopamine receptor (D1-4)
- Serotonin receptor (5 HT1,2,5,6,7)
- Angiotensinreceptor
- Endothelinreceptor

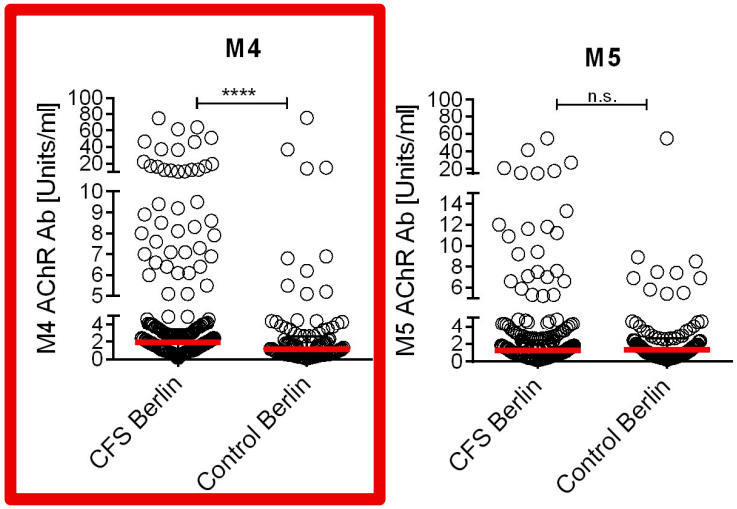
Elevated autoantibodies against neurotransmitter receptors in 30% of CFS/ME patients

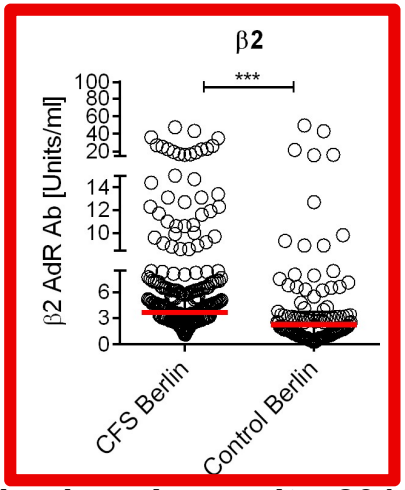
Acetylcholine receptor antibodies (M1-5)

Adrenergic receptor-antibodies (ß1+2)







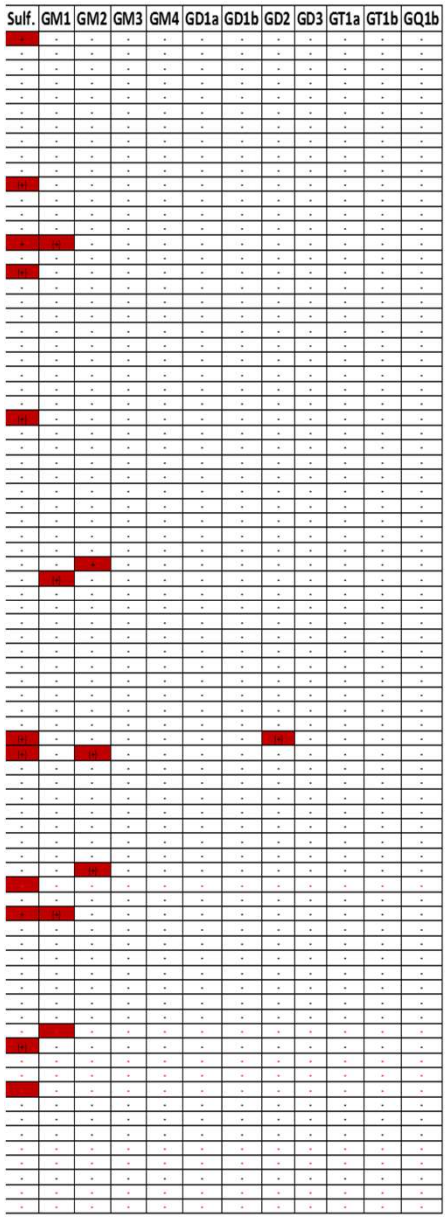


Brain, Behaviour, Immunity, 2016

Antibodies against several subtypes of gangliosides – own data*

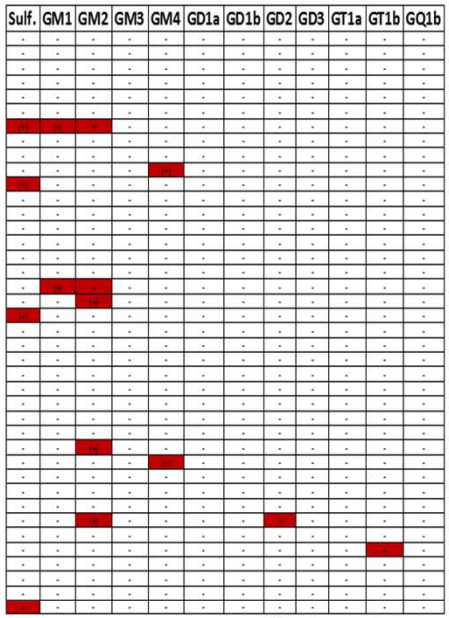
* in collaboration with
Prof . Roggenbuck
Generic Assays

CFS patients

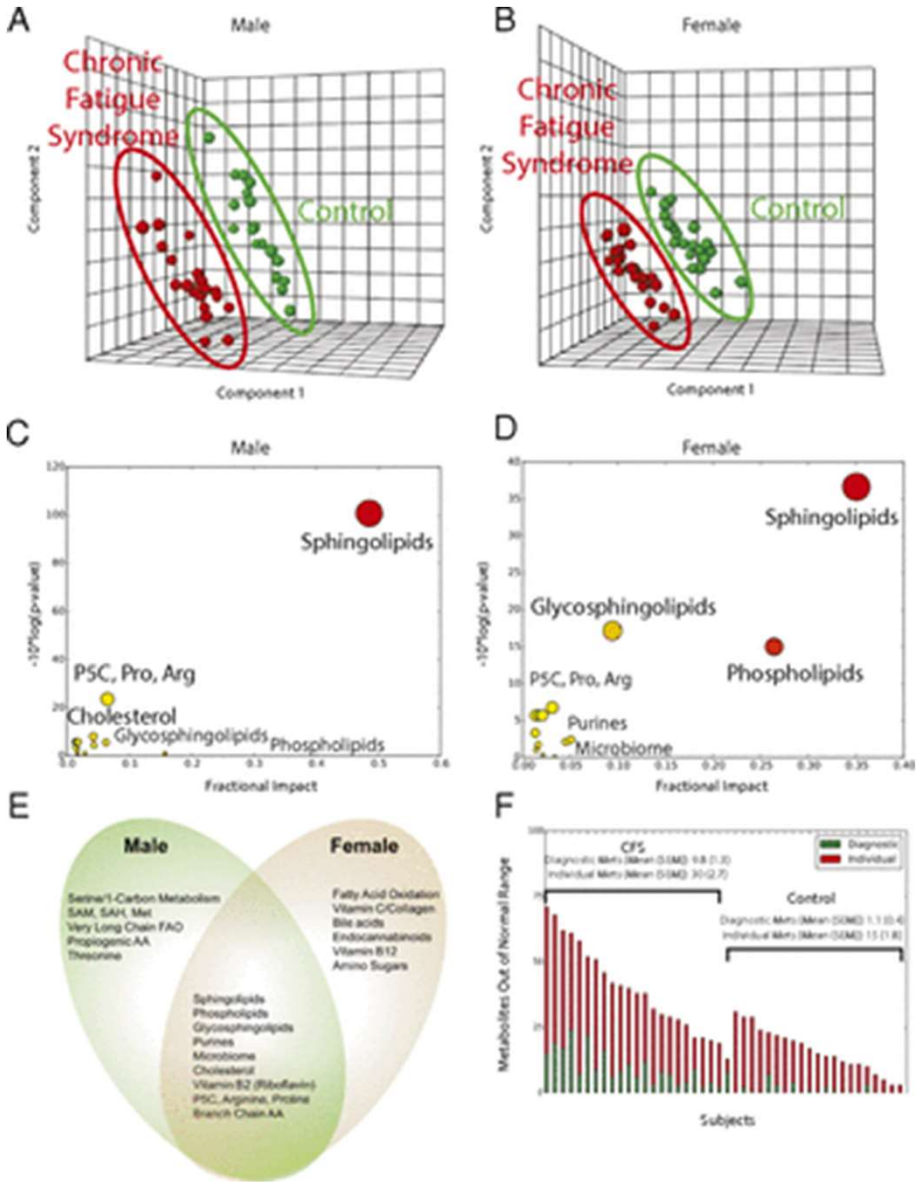


18.5% positive

Healthy controls



25% positive


A
Male
Chronic Fatigue Syndrome
Control
Component 2
Component 1
B
Female
Chronic Fatigue Syndrome
Control
Component 2
Component 1
C
Male
Sphingolipids
PSC, Pro, Arg
Cholesterol
Glycosphingolipids
Phospholipids
-10*log(p-value)
Fractional Impact
D
Female
Sphingolipids
Glycosphingolipids
Phospholipids
PSC, Pro, Arg
Purines
Microbiome
-10*log(p-value)
Fractional Impact
E
Male
Female
Serine/1-Carbon Metabolism
SAM, SAH, Met
Very Long Chain FAO
Propiogenic AA
Threonine
Fatty Acid Oxidation
Vitamin C/Collagen
Bile acids
Endocannabinoids
Vitamin B12
Amino Sugars
Sphingolipids
Phospholipids
Glycosphingolipids
Purines
Microbiome
Cholesterol
Vitamin B2 (Riboflavin)
PSC, Arginine, Proline
Branch Chain AA
F
CFS
Control
Diagnostic
Individual
Metabolites Out of Normal Range
Subjects

Data on microRNAs as biomarker

RESEARCH ARTICLE

MicroRNAs hsa-miR-99b, hsa-miR-330, hsa-miR-126 and hsa-miR-30c: Potential Diagnostic Biomarkers in Natural Killer (NK) Cells of Patients with Chronic Fatigue Syndrome (CFS)/ Myalgic Encephalomyelitis (ME)

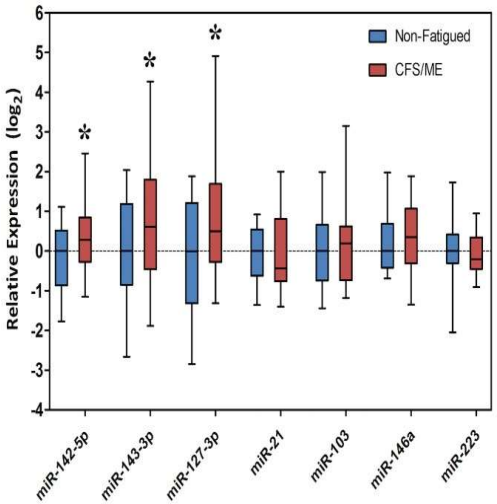
Robert D. Petty[1,2]*, Neil E. McCarthy[3], Rifca Le Dieu[2], Jonathan R. Kerr[1,4]

2016, Plos One

PLoS One. 2014 Sep 19;9(9):e102783. doi: 10.1371/journal.pone.0102783. eCollection 2014.

High-throughput sequencing of plasma microRNA in chronic fatigue syndrome

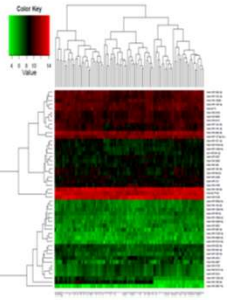
Brenu EW[1], Ashton KJ[2], Batovska J[2], Staines DR[3], Marshall-Gradisnik SM[1].



We found no strong difference in microRNA expression:

PAXgene whole blood sa… Agilent Technologies …t

	mean		FC	$\log_2$ FC	*t*-test		AUC
	group 1	group 2			raw *p*	adj. *p*	
hsa-miR-144-5p	9.42	9.97	0.68	-0.55	1.26E-04	4.31E-03	0.70
hsa-miR-126-3p	10.35	10.80	0.73	-0.46	6.33E-06	8.36E-04	0.74
hsa-miR-26b-5p	11.35	11.80	0.73	-0.45	5.21E-06	8.36E-04	0.74
hsa-miR-374b-5p	9.39	9.80	0.76	-0.40	1.62E-05	1.33E-03	0.75
hsa-let-7f-5p	12.43	12.80	0.77	-0.38	1.05E-04	3.90E-03	0.70
hsa-miR-454-3p	7.03	7.39	0.78	-0.36	6.12E-04	1.47E-02	0.66
hsa-let-7d-5p	11.95	12.29	0.79	-0.34	2.70E-06	8.36E-04	0.76
hsa-miR-98-5p	6.23	6.56	0.79	-0.33	5.41E-04	1.38E-02	0.69
hsa-miR-100-5p	7.24	6.91	1.25	0.32	4.47E-01	6.88E-01	0.47
hsa-let-7g-5p	12.61	12.93	0.80	-0.32	1.99E-05	1.36E-03	0.72
hsa-let-7a-5p	13.98	14.30	0.80	-0.32	6.49E-05	3.79E-03	0.70
hsa-miR-363-3p	11.77	12.09	0.80	-0.32	8.18E-06	8.36E-04	0.79
hsa-miR-21-5p	10.81	11.12	0.81	-0.31	2.26E-04	7.11E-03	0.71
hsa-miR-148a-3p	8.01	8.31	0.81	-0.30	3.74E-03	5.67E-02	0.67
hsa-miR-20b-5p	10.70	11.00	0.81	-0.30	1.63E-03	2.90E-02	0.67
hsa-miR-151a-5p	10.99	11.27	0.82	-0.28	7.79E-05	3.90E-03	0.72
hsa-miR-374a-5p	8.17	8.44	0.83	-0.27	6.80E-03	7.94E-02	0.65
hsa-miR-4730	7.08	6.81	1.21	0.27	2.86E-02	1.98E-01	0.37
hsa-miR-6716-3p	6.93	6.66	1.21	0.27	2.61E-02	1.87E-01	0.36
hsa-miR-424-5p	5.19	5.46	0.83	-0.27	2.41E-03	4.10E-02	0.67
hsa-miR-451b	6.69	6.42	1.20	0.27	2.40E-02	1.76E-01	0.36
hsa-miR-126-5p	4.85	5.11	0.83	-0.27	8.61E-04	1.85E-02	0.68
hsa-miR-101-3p	8.18	8.44	0.83	-0.26	1.66E-02	1.38E-01	0.61
hsa-miR-199a-3p	5.84	6.09	0.84	-0.25	1.08E-02	1.08E-01	0.63
hsa-miR-29c-3p	9.05	9.29	0.85	-0.24	4.46E-03	6.31E-02	0.67
hsa-miR-24-3p	10.19	10.42	0.85	-0.24	1.31E-03	2.55E-02	0.70
hsa-miR-15a-5p	12.38	12.61	0.85	-0.24	2.49E-04	7.27E-03	0.68
hsa-miR-301a-3p	5.52	5.75	0.85	-0.23	5.49E-03	7.24E-02	0.66
hsa-miR-183-5p	8.78	9.02	0.85	-0.23	6.92E-02	2.78E-01	0.61
hsa-miR-181a-5p	8.04	8.27	0.86	-0.22	1.40E-02	1.25E-01	0.66



Group 1 healthy
Group 2 CFS
FC (healthy rel. to

Validation experiments failed to reproduce expression levels of microRNAs by single tube PCRs using miRCURYTM LNA m… RNA PCR …

EXIQON
Seek Find Verify

Biomarker in CFS

Biomarkers	References
>IL-10, IFNγ, TNFα by PHA stimulated lymphocytes; >CD4+CD25+ T cells expressing FoxP3 and VPACR2; <cytotoxic activity of NK and CD8+T cells; <granzyme and >perforin by gene expression in CFS/ME compared to HC at rest.	Brenu, et al., 2011
>IL-4, IL-5, IL-12, LTα, IL-1α, IL-1β, IL-6; <IL-8, IL-13, IL-15; ≥IL-2, IFNγ, IL-17, IL-23, TNFα in plasma of CFS/ME compared to HC at rest.	Fletcher, et al., 2009
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<Perforin in NK cells and CD8+T cells by quantitative flow cytometry. CFS/ME compared to HC at rest	Maher, et al., 2005
<Perforin by gene expression in GWI compared to HC at VO_2Max in exercise challenge	Whistler, et al, 2009
<NK cell cytotoxicity; < plasma dipeptidyl peptidase IV;>T-cell activation. CFS/ME compared to HC at rest	Fletcher, et al., 2010a
In CFS/ME compared to HC: absence of significant increase in IL-6 & TNFα following exercise challenge	Jammes, et al., 2009
IL-1β, IL-12, IL-6, IL-8, IL-10, and IL-13 elevated at 8 hrs post exercise in subjects showing symptom flair at 48 hrs.	White, et al, 2010
>NPY in CFS/ME subjects compared to HC at rest;	Fletcher, et al., 2010b
no exercise related change for NPY, IL-6, IL-10, IL12, TNFα in CFS/ME but > in HC	Harvey, et al., 2011
<Serum vitamin E, a marker for oxidative stress in CFS/ME compared to HC at rest	Miwa & Fujita, 2010
Exercise related <plasma F(2)-isoprostanes (marker of oxidative stress); No effect of exercise on plasma IL-6 or sIL-6R in CFS/ME or HC.	Robinson, et al., 2010
In most CFS/ME but not in HC, exercise >transcription for most sensory and adrenergic receptors and one cytokine which correlated with fatigue and pain.	Light, et al, 2012
Metabolic syndrome predictors elevated in CFS/ME compared to HC at rest.	Maloney, et al, 2010
Increased lactate levels in ventricular cerebrospinal fluid of CFS/ME compared to HC at rest.	Murrough et al. 2010
Significant deficiencies in mitochondrial function in CFS/ME compared to HC at rest.	Myhill et al., 2009
Quantitative proteomics using high resolution mass spectrometry of CSF, unique patterns associated with CFS compared to HC and Lyme disease at rest.	Schutzer, et al., 2011
Unique CFS/ME spinal fluid proteome of 60 proteins when compared to HC and GWI. The CFS/ME and GWI patients shared 20 unique proteins at rest.	Baraniuk et al 2005
> CRP, >8-iso-prostaglandin F(2 alpha) isoprostanes in CFS/ME compared to HC at rest	Spence, et al, 2008
<LPS induced pro-inflammatory cytokines under psychological stress in CFS/ME compared to HC at rest	Gaab et al, 2005
> CRP in CF cases not meeting the CFS/ME definition; no difference between CFS/ME and HC at rest	Raison et al, 2009
Abnormal pattern of cortisol over 24 hours associated with elevated fatigue.	Torres-Harding, et al 2009
<Cortisol levels and flattened diurnal release of cortisol) associated with a poorer response to CBT in CFS/ME.	Roberts, et al., 2010
Variations in the 5' region of NR3C1 (glucocorticoid receptor gene) in CFS/ME compared to HC at rest.	Rajeevan et al., 2007
HPA axis dysfunction in CFS/ME compared to HC at rest.	Papadopoulos & Cleare, 2011
HPA axis dysfunction CFS/ME compared to HC at rest.	Ben-Zvi, et al., 2009
No evidence for biomarkers using gene expression in a twin study.	Byrnes et al., 2009
Significant evidence for a heritable contribution to predisposition to CFS/ME.	Albright, et al, 2011
Gene expression revealed 'CFS signature genes'.	Kerr, et al., 2008
Reassessment of 'CFS signature genes' failed to confirm predictive ability.	Frampton, et al., 2011

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Biomarkers for chronic fatigue.

Klimas NG[1], Broderick G, Fletcher MA.

	Friedman Test Result: Significant Values ($p < .05$) in Bold	
	CFS/ME (N=23)	HC (N=34)
%CD26+CD2+ (T & NK cells)	**<.000 (↓)**	**<.000 (↓)**
rMolCD26/CD2+ (T & NK cells)	**0.004 (↓)**	**<.000 (↑)**
NPY (pMol/L plasma)	0.436	**<.000 (↑)**
IL-6 (pg/ml plasma)	0.607	**0.008 (↑)**
IL-10 (pg/ml plasma)	0.857	**0.001 (↑)**
IL-12p70 (pg/ml plasma)	0.354	**0.002 (↑)**
TNFα (pg/ml plasma)	0.624	**0.007 (↑)**
rMolPerforin/NK cell	**0.012 (↑)**	**<.000 (↑)**
NKCC (%)	**0.023 (↑)**	**0.001 (↑)**

Chronic Fatigue Syndrome: The **Current Status** and Future Potentials of Emerging Biomarkers.

Fischer DB, William AH, Strauss AC, Unger ER, Jason L, Marshall GD Jr, Dimitrakoff JD.

Fatigue. 2014 Jun 1;2(2):93-109.

Immunological Biomarkers

Biomarker	**Findings**
Cytokine markers	High levels of TNF-α, IL-1, PMN-elastase, lysozyme, and serum neopterin Increased levels of IL-10. Decreased IFN-γ/IL-10 ratio T_H2 shift
NK surface markers	CD26 and CD69 reduced on CD8+ T cells and NK cells
Humoral immunity	Rituximab led to symptom improvement in patients with CFS
Inflammatory characteristics	Increased histone deacetylase activity and lower total antioxidant power. Decreased plasma cortisol. Increased plasma dehydroepiandrosterone.
Cellular cytotoxic findings	VPACR2 highly expressed on T cells