EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

European Network on Myalgic Encephalomyelitis/Chronic Fatigue Syndrome
COST Action - CA15111

8 February 2018

Prof. Carmen Scheibenbogen
& Dr. Franziska Sotzny

Institute for Medical Immunology,
Charité Universitätsmedizin

3. Continuation of Work Group No 2 meeting

Presentation of review projects – Autoimmunity

Dr. Franziska Sotzny

Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome – evidence for an autoimmune disease

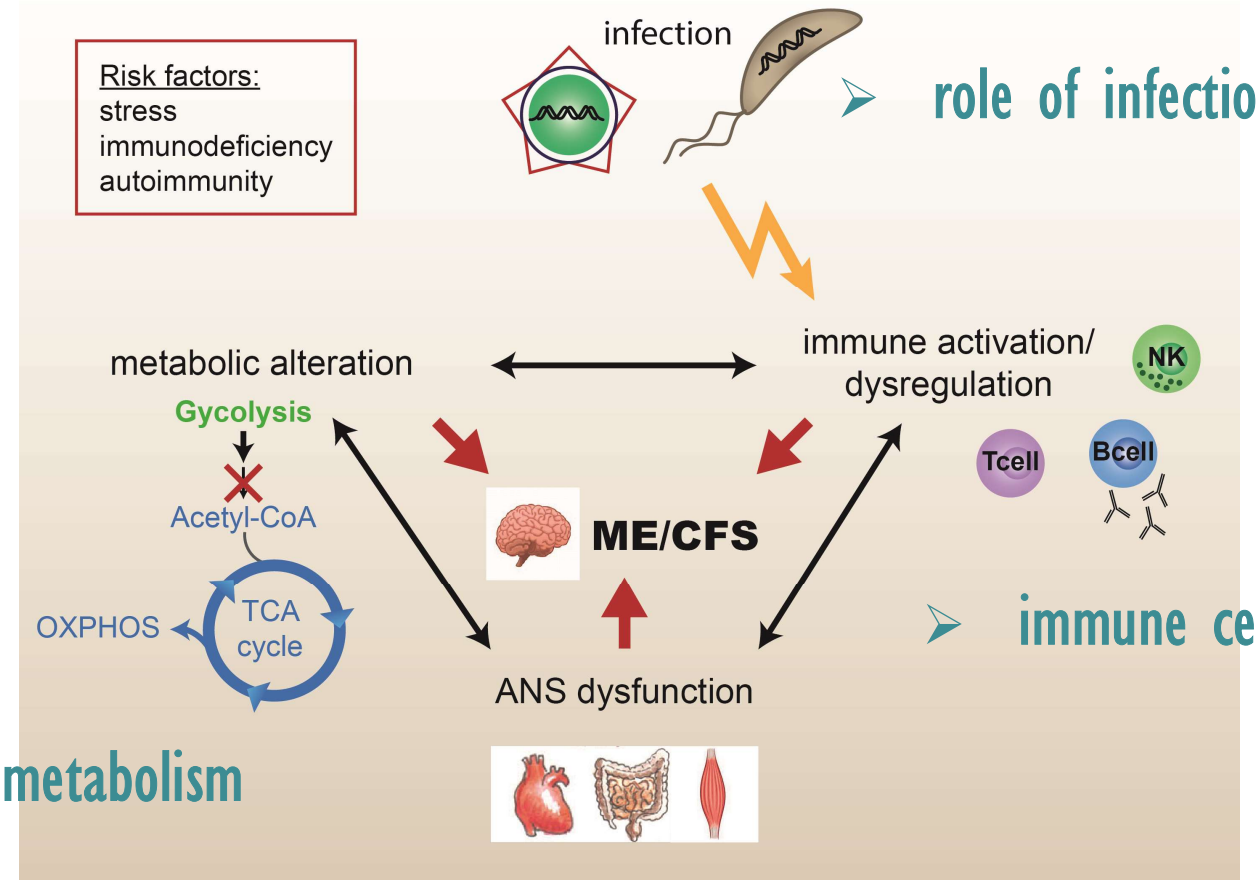
Autoimmunity Reviews

Co-Editors-in-Chief: Y. Shoenfeld, M.E. Gershwin, MD

Impact Factor: **8.961**

Authors: *Franziska Sotzny, Julià Blanco, Enrica Capelli, Jesús Castro-Marrero, Sophie Steiner, Modra Murovska, Carmen Scheibenbogen*

ME/CFS – evidence for an autoimmune disease



➢ role of infection

➢ immune cell alteration

➢ energy metabolism

ME/CFS – evidence for an autoimmune disease

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- autoantibodies as markers

Autoantigen	Cohorts of patients/control (n)	Autoantibody positive patients/control (%)	Refs.
Nuclear structures			
ANA	60/51 225 60 60 139/149	68/15 23 57 68 7/5 (BioPlex ANA screen) 4/6 (IIF)	[51] [53] [54] [55] [57]
Nuclear envelope	60/51 60/30	52/2 52/3	[51] [55]
Reticulated speckles	60/30	25/0	[55]
68/48 kDa protein	114/37	13/0	[52]
dsDNA	81	12	[56]
Membrane structures			
Phospholipids	42	38	[58]
Cardiolipin	26 40 81	92 (IgM) 95 (IgM) 4	[59] [60] [56]
Phosphatidylserine	81	5	[56]
Gangliosides	42/100 (FM)	43	[58]
Neurotransmitter receptors and neurotransmitter			
M AChR	5/11	PET: binding to brain M AChR in ME/CFS	[61]
M1 AChR	60/30	53/0	[54]
M3/4 AChR and β2-AdR	268/108	significantly elevated compared to healthy controls	[53]
5-HT	42 81	62 9	[58] [56]
Other autoantibodies			
Cytoplasmic intermediate filaments	60/30	35/13	[55]
dUTPase	55/151	15/5	[22]
Neopitopes formed by oxidative or nitrosative damage	14/11 16/17	significantly elevated compared to healthy controls (IgM titers)	[66] [67]

ME/CFS – evidence for an autoimmune disease

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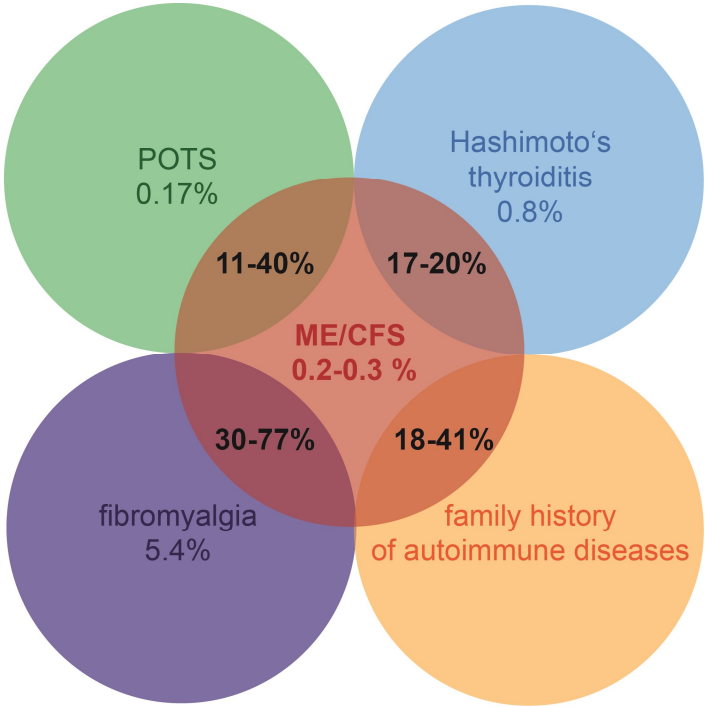
➢ comorbidity



➢ soluble markers

➢ autoantibodies

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➢ genetic variants

ME/CFS – evidence for an autoimmune disease

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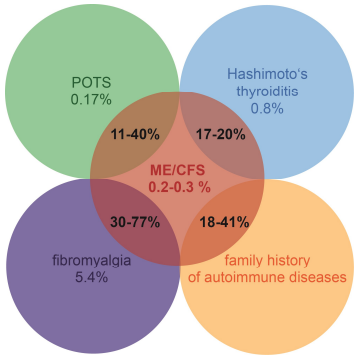
➢ comorbidity

➢ soluble markers



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➢ genetic variants

➢ therapies

Dosage	Study design	Patients (n)	Evaluation	Outcome	Refs.
Intravenous IgG					
1g/kg/m² 6x	RCT	28	FI & SR	No difference	[133]
2g/kg/m² 3x	RCT	49	FI & SR	Follow-up m3: 43% vs. 12%	[134]
0.5g/1g/2g/kg/m² 3x	RCT	99	FI & SR	No difference	[135]
1g/kg/m² 3x	RCT	70 (adolescents)	FI	Follow-up m6: 72% vs. 44%	[136]
Rituximab					
500 mg/m² 2x	RCT	30	FI & SR	Improvement 67% vs. 13%	[131]
500 mg/m² 6x	single arm	29	FI & SR	Improvement 62%	[129]
Ongoing Trials					
Cyclophosphamide (Endoxan®)					Fluge *et al.*, unpub.
Immunoadsorption					Scheibenbogen *et al.*, unpub.

Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome – evidence for an autoimmune disease

Franziska Sotzny [**], Julià Blanco, Enrica Capelli, Jesús Castro-Marrero, Sophie Steiner, Modra Murovska, Carmen Scheibenbogen[*] on behalf of European Network on ME/CFS (EUROMENE)

Autoimmunity Reviews

* Carmen.Scheibenbogen@charite.de
** Franziska.Sotzny@charite.de

4. Biomarker Studies

Landscape Study and Update

Dr. Franziska Sotzny

Scheibenbogen et al. J Transl Med (2017) 15:162
DOI 10.1186/s12967-017-1263-z

Journal of Translational Medicine

REVIEW Open Access

The European ME/CFS Biomarker Landscape project: an initiative of the European network EUROMENE

Carmen Scheibenbogen[1*], Helma Freitag[1], Julià Blanco[3,4], Enrica Capelli[5,6], Eliana Lacerda[7], Jerome Authier[8], Mira Meeus[9,10,11], Jesus Castro Marrero[12], Zaiga Nora-Krukle[2], Elisa Oltra[13,14], Elin Bolle Strand[15,16], Evelina Shikova[17], Slobodan Sekulic[18] and Modra Murovska[2]

- 2012 - 2016
- 39 studies were identified:
  - 15 immune markers
  - 5 infection markers
  - 4 neurological markers
  - 15 metabolic markers

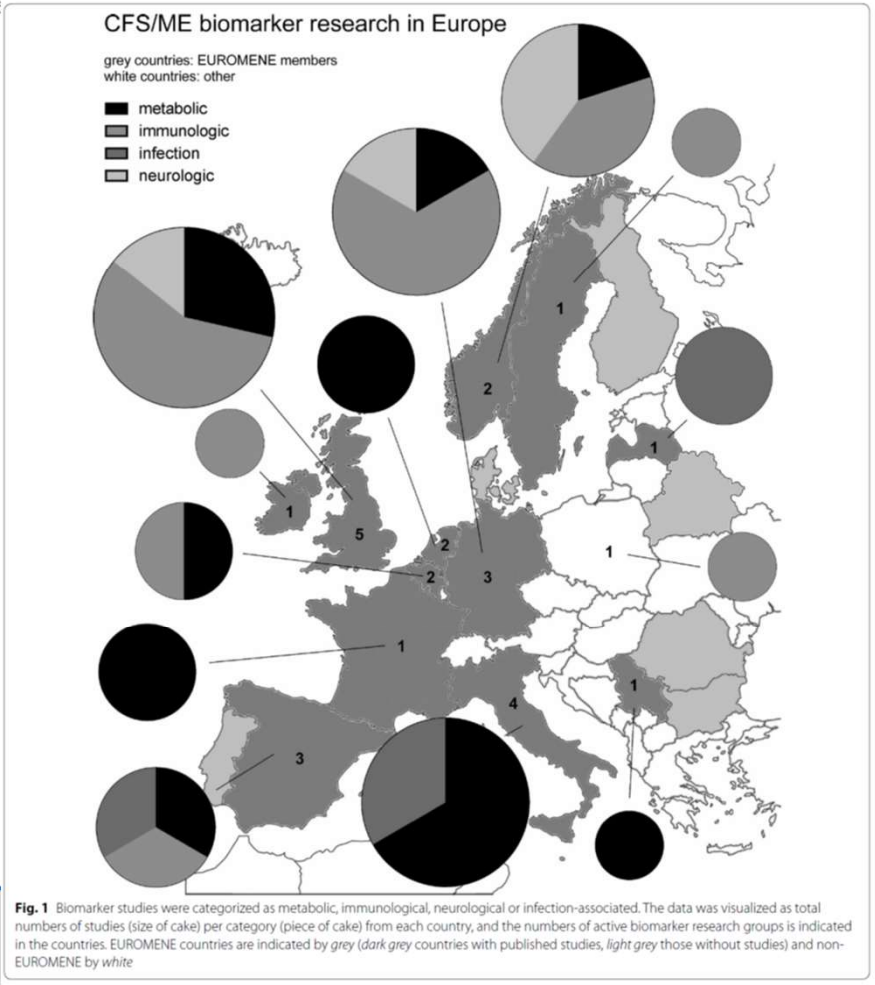

Fig. 1 Biomarker studies were categorized as metabolic, immunological, neurological or infection-associated. The data was visualized as total numbers of studies (size of cake) per category (piece of cake) from each country, and the numbers of active biomarker research groups is indicated in the countries. EUROMENE countries are indicated by *grey* (*dark grey* countries with published studies, *light grey* those without studies) and non-EUROMENE by *white*

ME/CFS Biomarker Landscape project – UPDATE

- year 2017
- worldwide
- 14 studies were identified:
  - 7 immune marker
  - 3 immune & infection marker
  - 2 immune & metabolic marker
  - 2 metabolic marker

Country	Category	Reference
Germany	immunologic/ infection	Loebel *et al.*
Norway	immunologic immunologic Immunologic/ infection	Theorell et al. Nguyen et al. (a) Hanevik et al.
UK	metabolic	Tomas et al.
Australia	immunologic immunologic immunologic immunologic	Lidbury et al. Nguyen et al. (b) Nguyen et al. (c) Broadbent et al.
Canada	metabolic	de Vega et al.
USA	metabolic immunologic	Germain et al. Montoya et al.
…		

Antonia Berz

ME/CFS Biomarker Landscape project - potential immune and infection biomarkers

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- Ig & MBL
- neurotransmitter receptor autoantibodies
- TGFβ
- serum BAFF
- active HHV-6 & HHV-7/HHV-7 & B19 infection
- TRPM3
- Activin B
- mast cells
- EBV serology:
  EBNA6, dUTPase

4. Biomarker Studies

Selection and validation of selected biomarkers

- first Charité data -

Dr. Franziska Sotzny

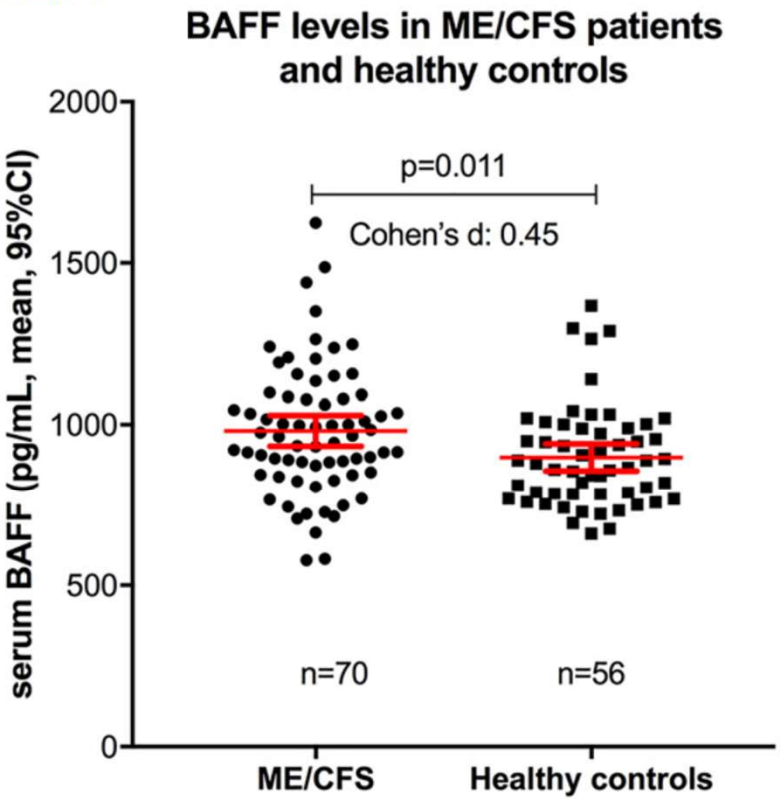
1. BAFF

Serum BAFF and APRIL Levels, T-Lymphocyte Subsets, and Immunoglobulins after B-Cell Depletion Using the Monoclonal Anti-CD20 Antibody Rituximab in Myalgic Encephalopathy/Chronic Fatigue Syndrome

Sigrid Lunde[1], Einar K. Kristoffersen[2,3], Dipak Sapkota[1,4], Kristin Risa[1], Olav Dahl[1,3], Ove Bruland[1,5], Olav Mella[1,3], Øystein Fluge[1]*

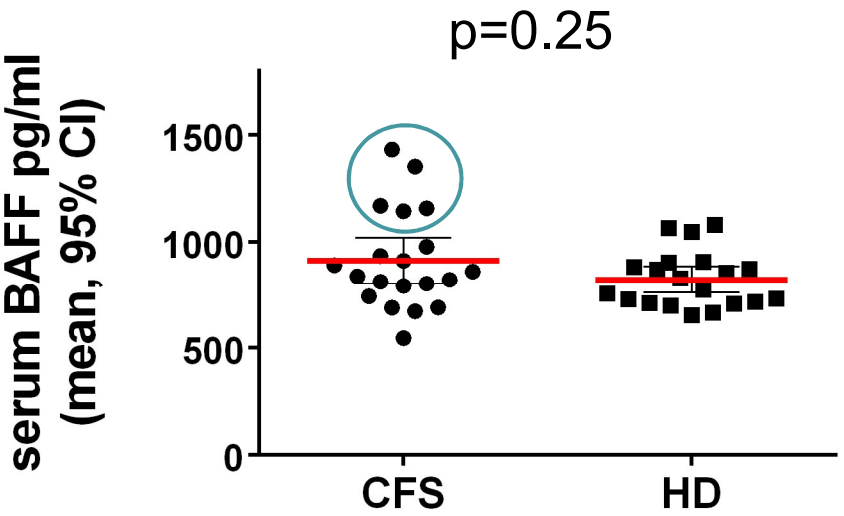
(2016)

- increase of the B lymphocyte activating factor (BAFF) concentration in ME/CFS patients vs controls



1. BAFF

- tendency of increased BAFF levels in ME/CFS patients vs controls determined by ELISA (n_{CFS}=20, n_{HD}=20)



Mann-Whitney test, two-tailed

2. Anti-ganglioside antibodies

Klein and Berg, 1995.

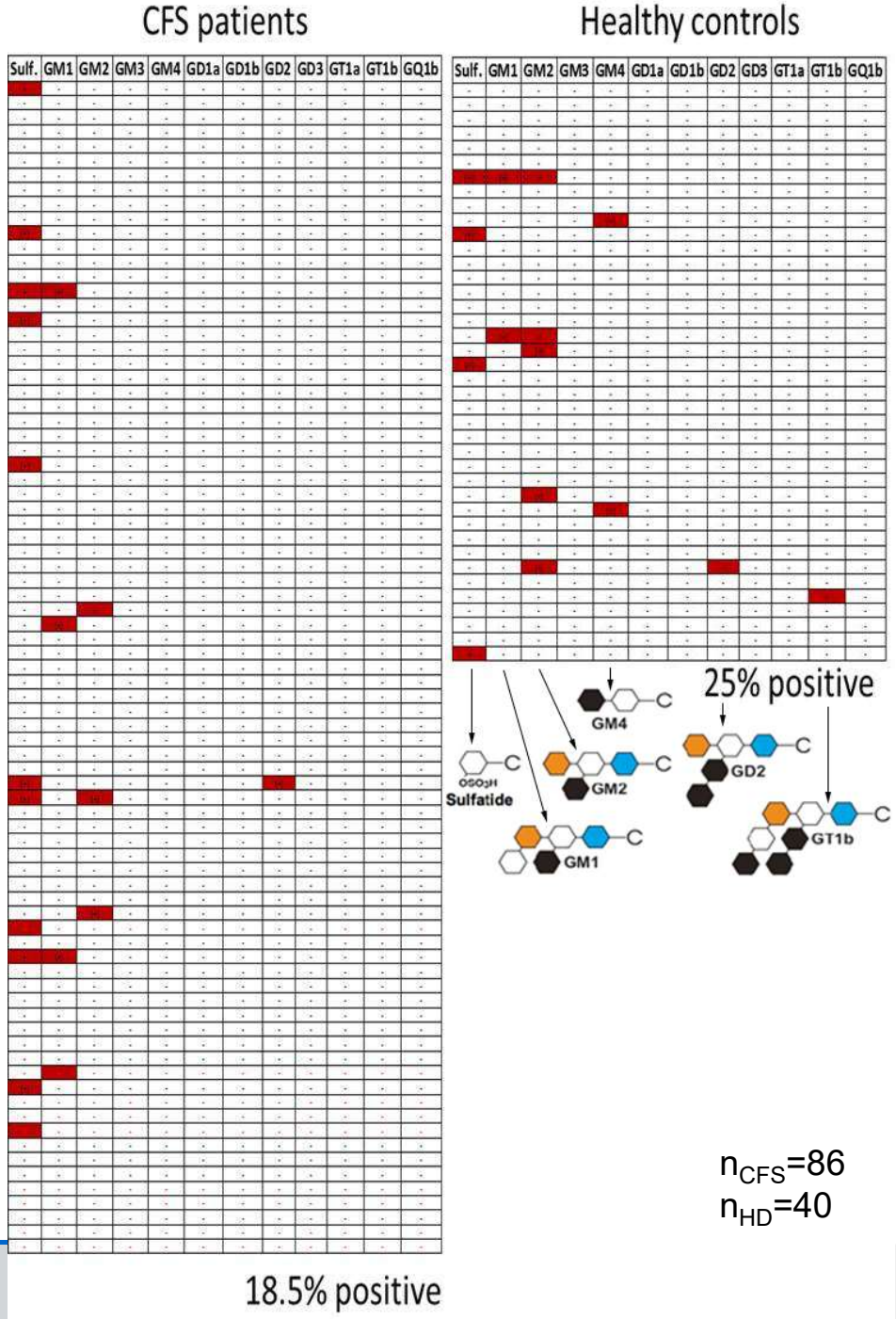
High incidence of antibodies to 5-hydroxytryptamine, **gangliosides** and phospholipids **in patients with chronic fatigue and fibromyalgia syndrome** and their relatives: evidence for a clinical entity of both disorders.

Eur J Med Res.

- Sera from 42 CFS patients were analysed by ELISA
- Results: **43 %** of the CFS patients had antibodies to gangliosides

2. Anti-ganglioside antibodies

- anti-Ganglioside dot assay was performed by the GA Generic Assays GmbH company



n_{CFS}=86
n_{HD}=40

3. CD26

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A Comparison of Immune Functionality in Viral versus Non-Viral CFS Subtypes

Nicole Porter[1,*], Athena Lerch[1], Leonard A. Jason[1], Matthew Sorenson[1], Mary Ann Fletcher[2], and Joshua Herrington[1] (2010)

- increase of CD26+CD2+ cells in CFS patients with viral onset and increase in CD26+CD2+CD8+ cells in ME/CFS patients

Lymphocyte Marker	Non-viral (*n*=62) M (SD)	Viral (*n*=46) M (SD)	*p*	*d*
CD2+ %pos	82.35 (6.98)	83.90 (5.51)		
CD2+ cells/µL	1702.24 (465.87)	1852.37 (644.16)		
CD2+ CD26+ % pos	55.36 (12.15)	**60.18 (8.68)** ↑	.024	-0.463
CD2+ CD26+cells/ µL	1140.24 (372.95)	1326.43 (469.89)	.029	-0.442
CD2+CD4+CD26 + %pos	41.10 (9.67)	48.08 (9.40)	.000	-0.731
CD2+CD4+CD26+ cells/ µL	846.90 (284.64)	1068.07 (423.02)	.003	-0.625
CD4+ CD26+ % pos	41.24 (9.69)	48.19 (9.34)	.000	-0.731
CD4+ CD26+ cells/µL	849.68 (285.38)	1070.37 (422.87)	.003	-0.623
CD2+CD8+CD26+ %pos	**12.07 (4.73)** ↑	**10.49 (4.64)** ↑		
CD2+CD8+CD26+ cells/µL	249.89 (118.40)	225.17 (108.77)		

3. CD26

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Biomarkers in Chronic Fatigue Syndrome: Evaluation of Natural Killer Cell Function and Dipeptidyl Peptidase IV/CD26

Mary A. Fletcher[1,2,3*], Xiao R. Zeng[1,2], Kevin Maher[1], Silvina Levis[1,2], Barry Hurwitz[3], Michael Antoni[3], Gordon Broderick[4], Nancy G. Klimas[1,2,3] (2010)

- increase of CD26+CD2+ cells and simultaneously a decrease in serum CD26 in ME/CFS patients vs controls

Table 1. Natural killer cell cytotoxicity and dipeptidyl peptidase IV/CD26 in chronic fatigue syndrome cases[a] compared to controls[b].

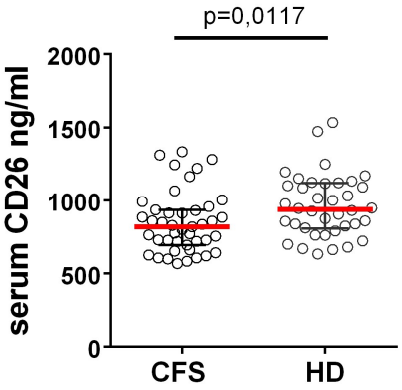
Variable	*Number of CFS Cases*	*Median (25–75th percentile)*	*Number of Healthy Controls*	*Median (25–75th percentile)*	*p*
NKCC%	176	12 (8–21)	230	28 (20–37)	.000
% CD26+CD2+ Cells	75	61 (55–66)	100	52 (47–59)	.000
sCD26 in Serum (ng/ml)	73	489 (396–643)	122	671 (496–871)	.000
rMol CD26/CD2+ Cell	77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

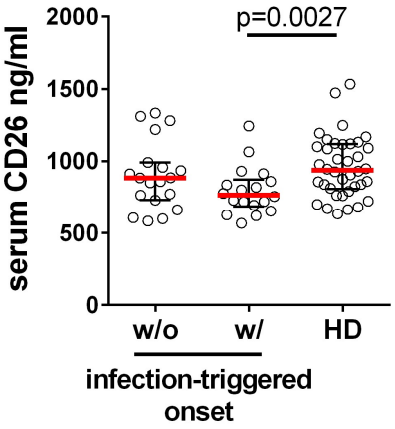
[a]>80% female, average age 48;
[b]>80% female, average age 47.

3. Serum CD26

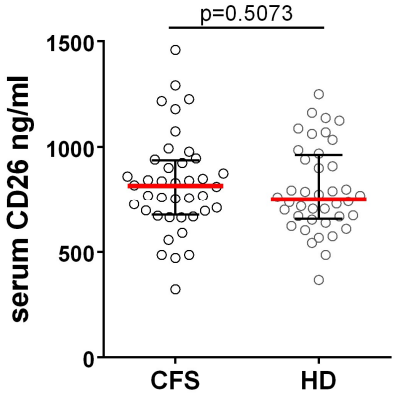
- <u>decrease in serum CD26</u> in ME/CFS patients vs controls determined by ELISA

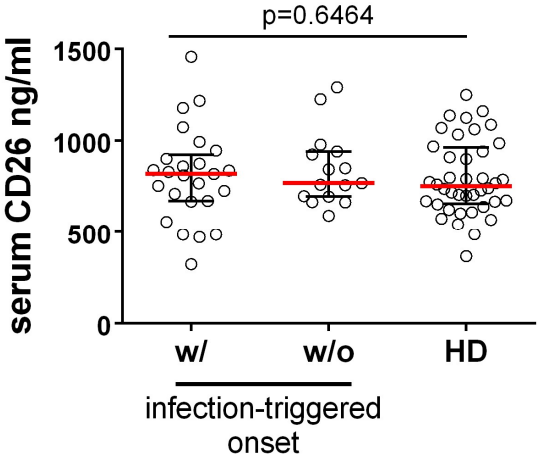
1st cohort:
n_{CFS}=43
n_{HD}=38





2nd cohort:
n_{CFS}=40
n_{HD}=40





Sandra Bauer

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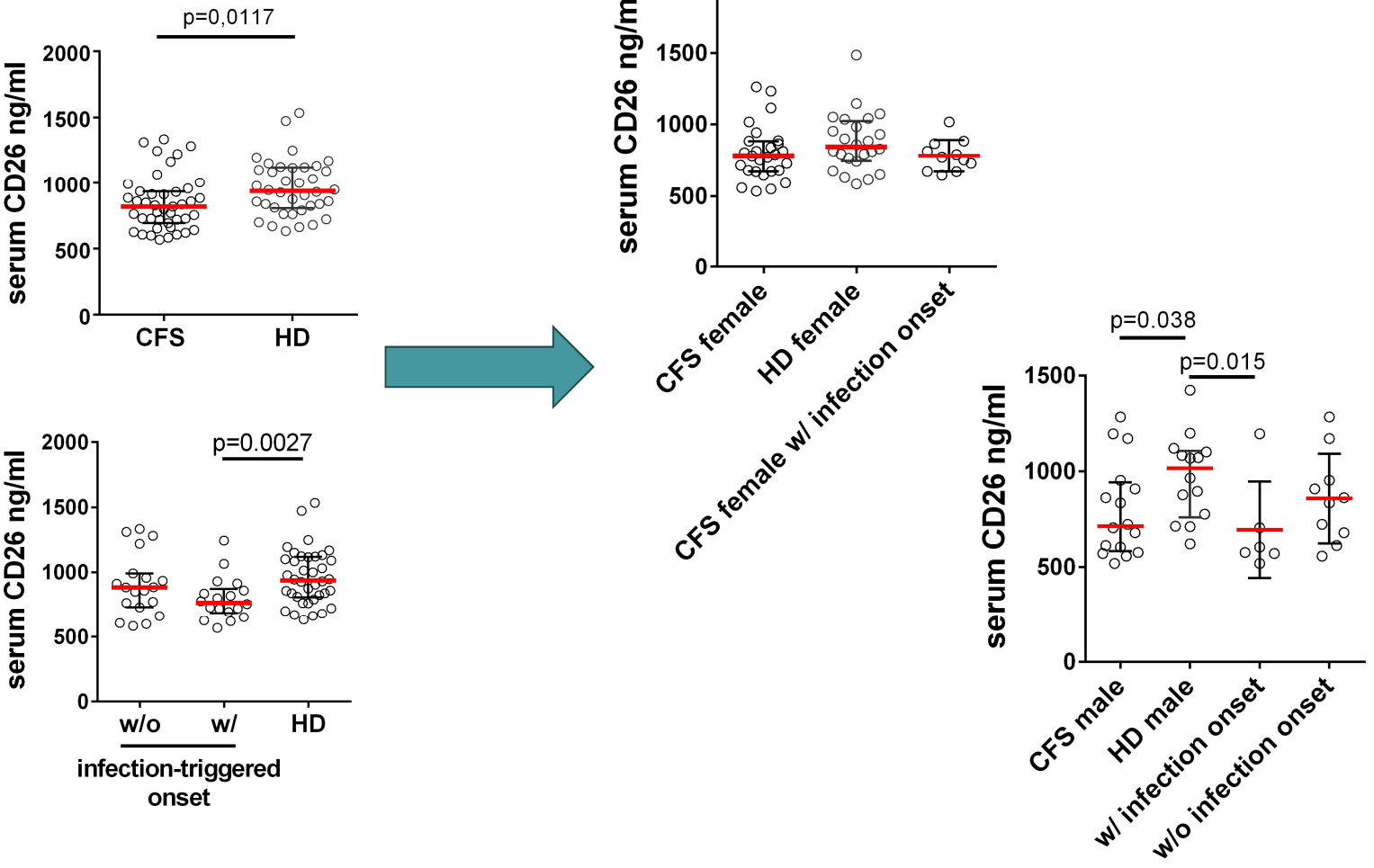
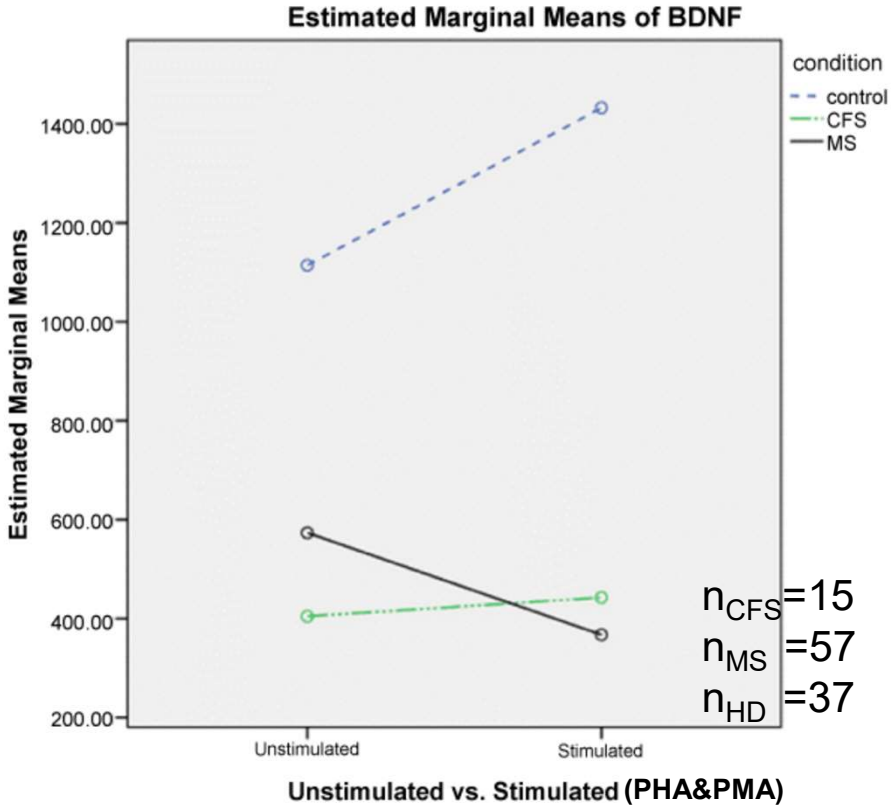


4. BDNF

Brain Derived Neurotrophic Factor is Decreased in Chronic Fatigue Syndrome and Multiple Sclerosis

Matthew Sorenson[1*], Leonard Jason[2], Jonna Peterson[3], Joshua Herrington[4], and Herbert Mathews[5] (2014)

- decreased BDNF production in PBMCs of ME/CFS patients vs controls
  - supernatant of 48 h PBMC culture was analysed by ELISA



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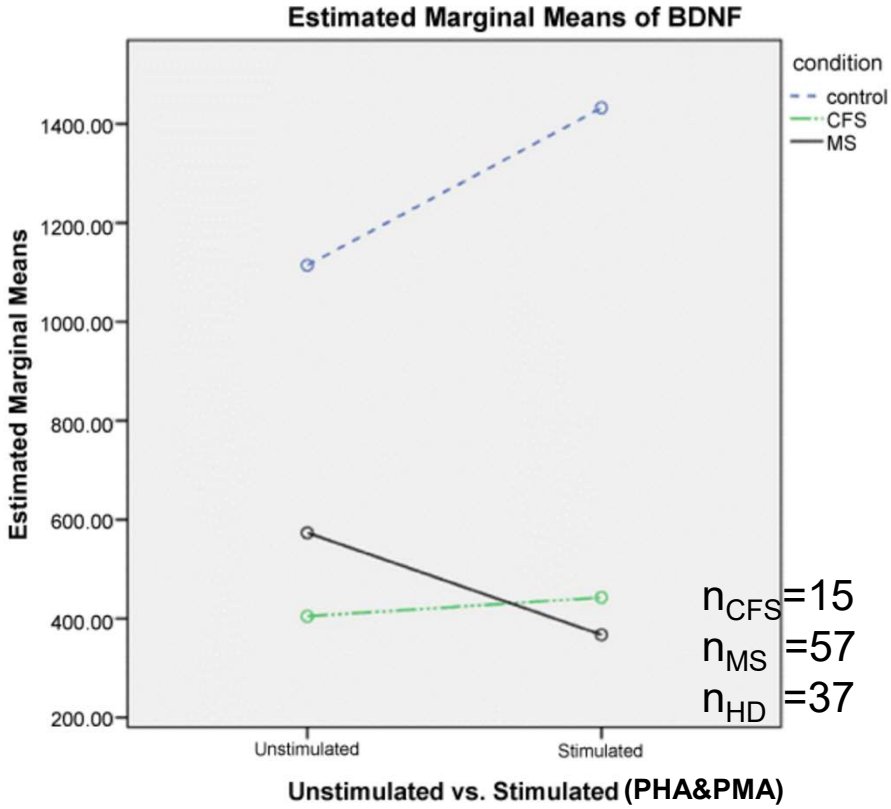
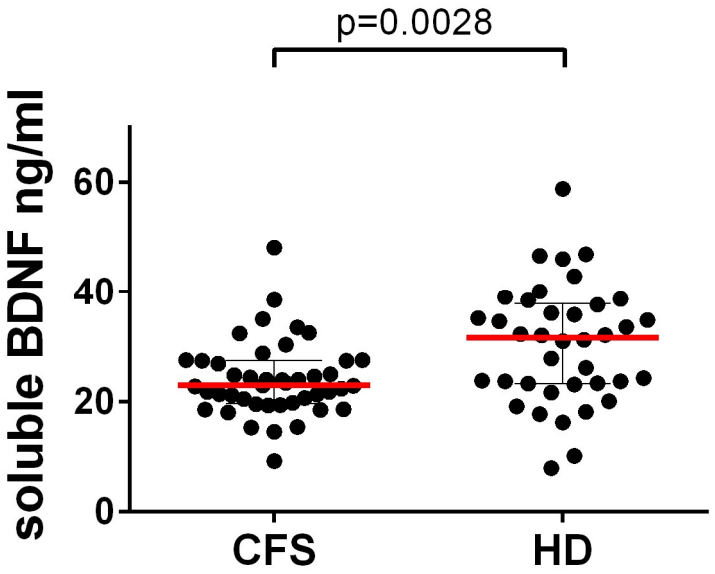
BDNF:

- formation of the developing nervous system
- contributes to neural plasticity through adulthood
- expressed in immune cells
- involved in the regulation of the energy homeostasis [Marosi and Mattson, 2014]



4. BDNF

- decreased serum BDNF levels in ME/CFS patients vs controls determined by ELISA (n_{CFS}=44; n_{HD}=38)



Mann-Whitney test, two-tailed

Sandra Bauer

4. Biomarker Studies

Selection and validation of selected biomarkers

- potential candidates -

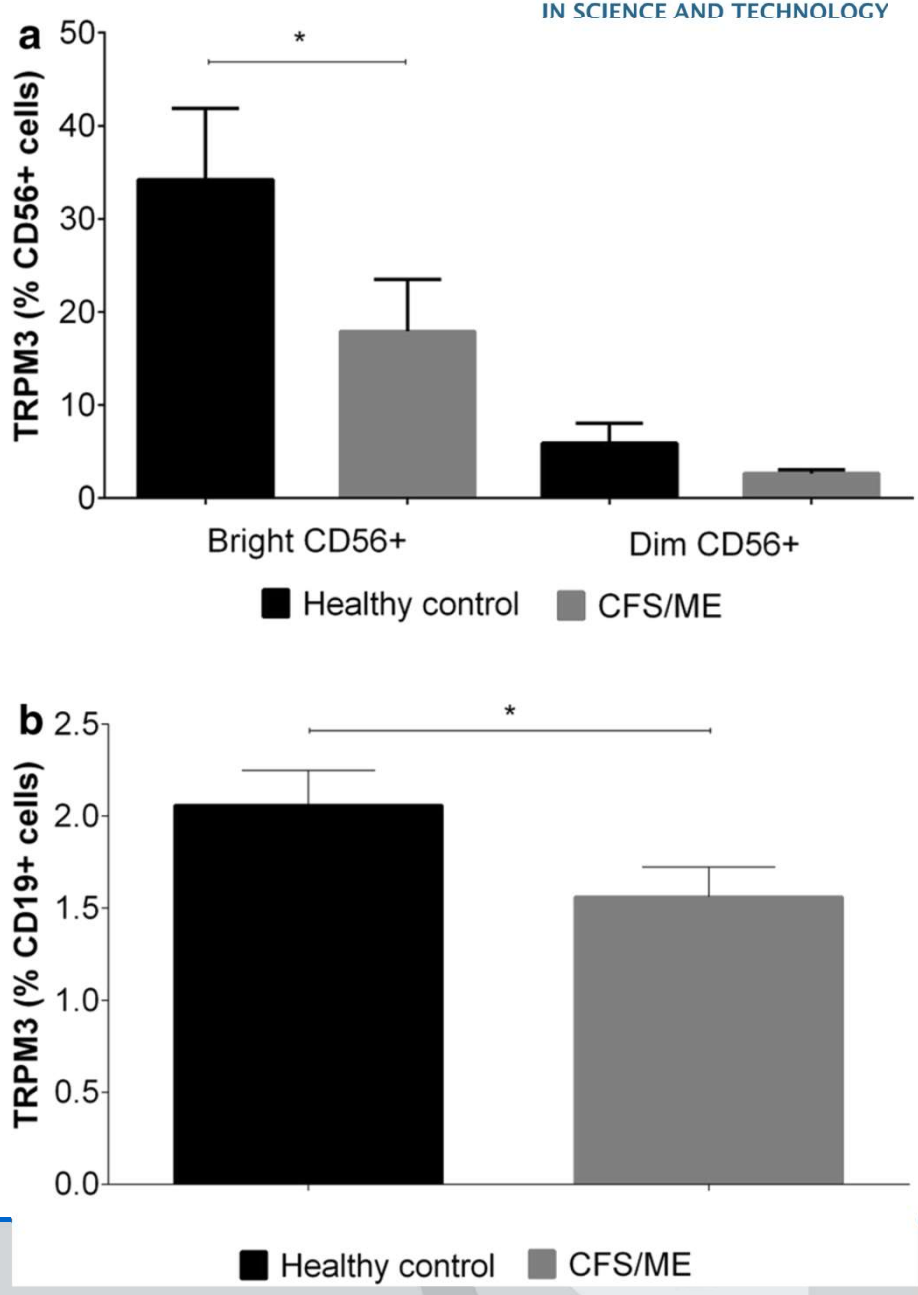
Dr. Franziska Sotzny

5. Candidates for biomarker validation - TRPM3

T. Nguyen, D. Staines … S. Marshall-Gradisnik; 2016

T. Nguyen, … D. Staines and S. Marshall-Gradisnik; 2017

- TRPM3 (transient receptor potential cation channel subfamily M member 3; calcium entry)
- significant reduction of TRPM3 surface expression on CD19+ B cells and CD56bright NK cells in CFS/ME (n = 17) compared with healthy controls (n= 19)
- 2nd study
  - confirmation of expression data
  - altered calcium signaling in NK cells of CFS/ME patients → altered downstream signaling → reduced cytotoxicity



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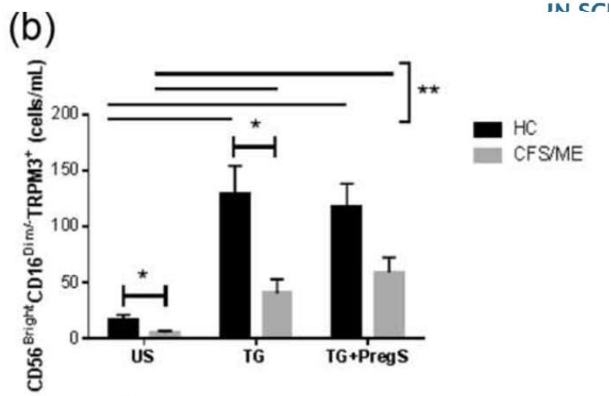
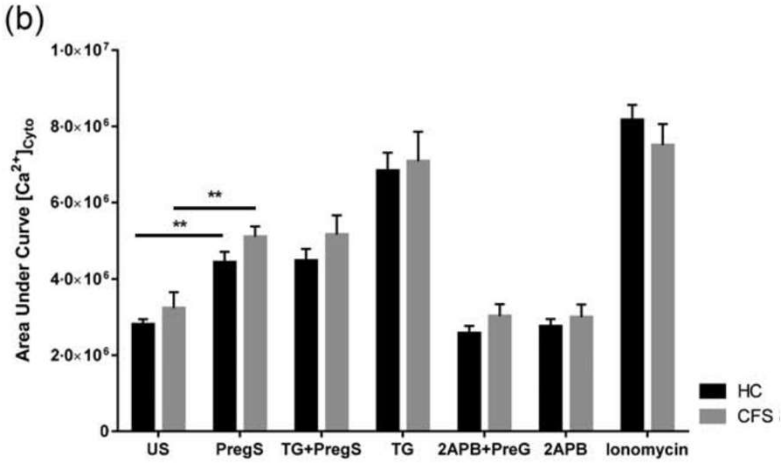
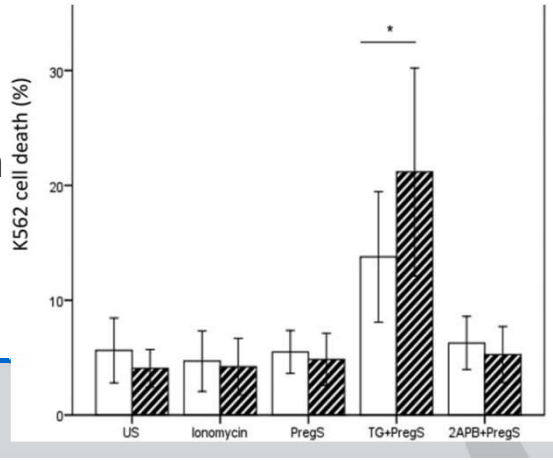






5. Candidates for biomarker validation - TRPM3

S. Marshall-Gradisnik, T. Huth… D. Staines ; 2016

S. Marshall-Gradisnik, S. Johnston … D. Staines ; 2016

- altered TRPM3 expression and/or activity may be explained by SNPs

<u>1st study</u>

- 678 SNPs from isolated NK cells - 21 TRP ion channel genes and 9 AChR genes

➢ identification of 11 SNPs for TRP ion channel genes (TRPC4, TRPC2, TRPM3, and TRPM8) in the ME/CFS group → 5 associated with TRPM3 [n_{CFS} = 39; n_{HD}=30]

<u>2nd study</u>

- 661 SNPs from B cells AChR and TRP variants and genotypes in B cells from CFS/ME patients

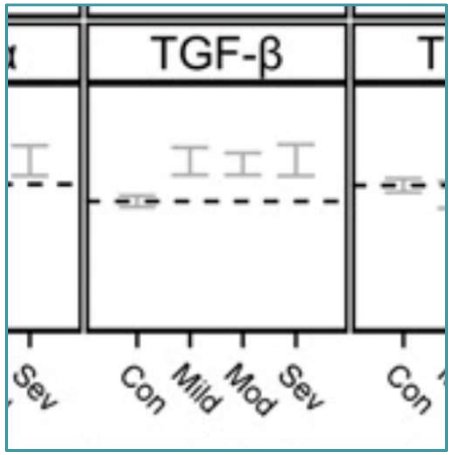
➢ 35 SNPs in the gene encoding mAChM3 and 4 SNPs in in the gene encoding TRPM3 [n_{CFS} = 11; n_{HD}=11]

5. Candidates for biomarker validation - immune marker: TGFβ

Blundell *et al*; 2015

Chronic fatigue syndrome and circulating cytokines: A systematic review

- 38 papers → cytokines in CFS/ME
- patients had significantly elevated TGF-β concentrations in 5 out of 8 (63%) studies (no other cytokines were present in abnormal concentrations in the majority of studies)



Montoya et al., 2017

n_{CFS}=192; n_{HD}=392

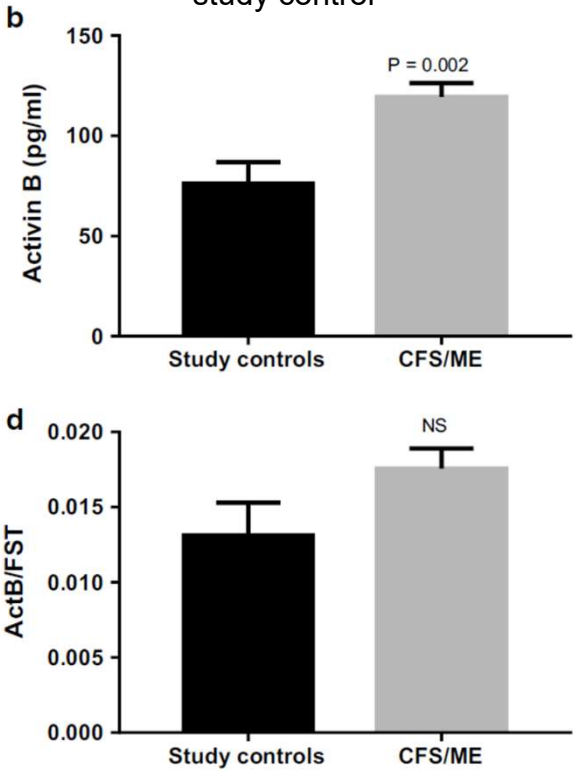
5. Candidates for biomarker validation
- immune marker: Activin B

Lidbury *et al*; 2017

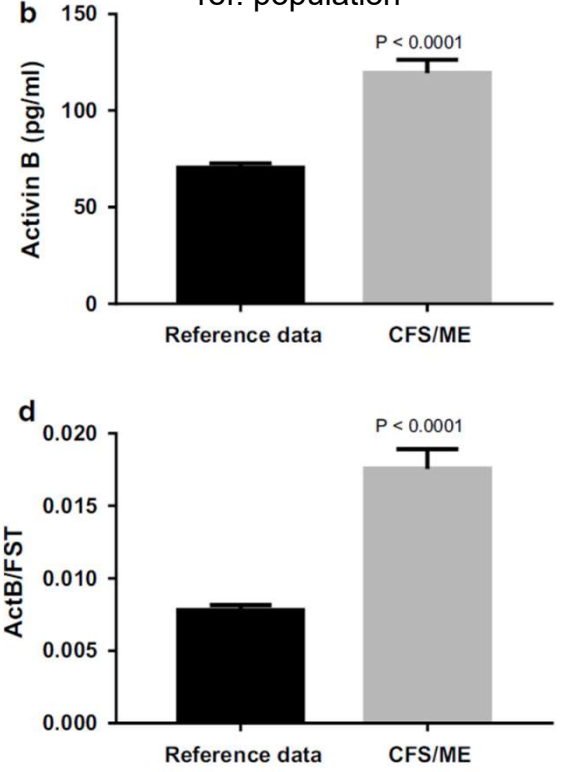
Activin B is a novel biomarker for chronic fatigue syndrome /myalgic encephalomyelitis (CFS/ME) diagnosis: a cross sectional study

n_{CFS} = 45 vs.

$n_{study\ control}$=17



$n_{ref.\ population}$=141



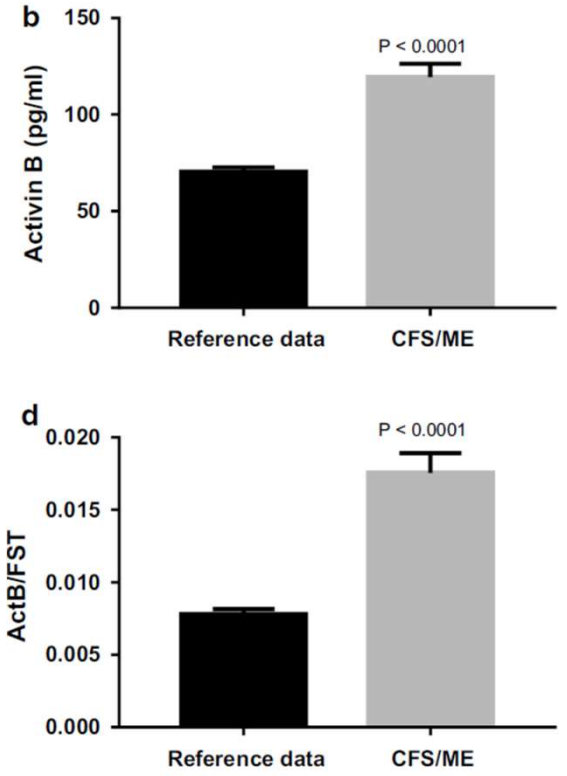
FST: follistatin

5. Candidates for biomarker validation - immune marker: Activin B

Lidbury *et al*; 2017

Activin B is a novel biomarker for chronic fatigue syndrome /myalgic encephalomyelitis (CFS/ME) diagnosis: a cross sectional study

- serum activin B levels for CFS/ME participants were significantly elevated when compared to the controls



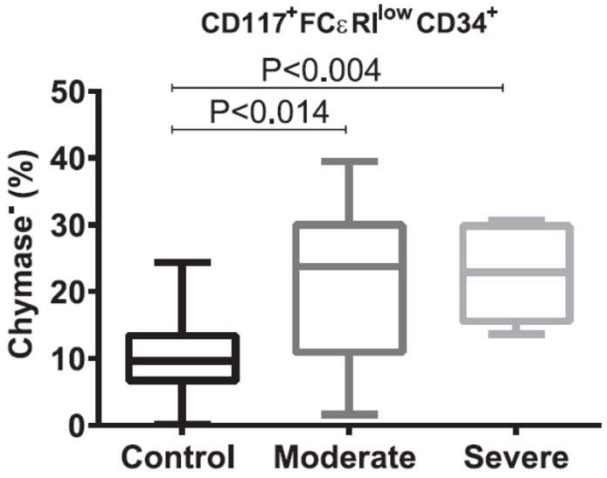
FST: follistatin

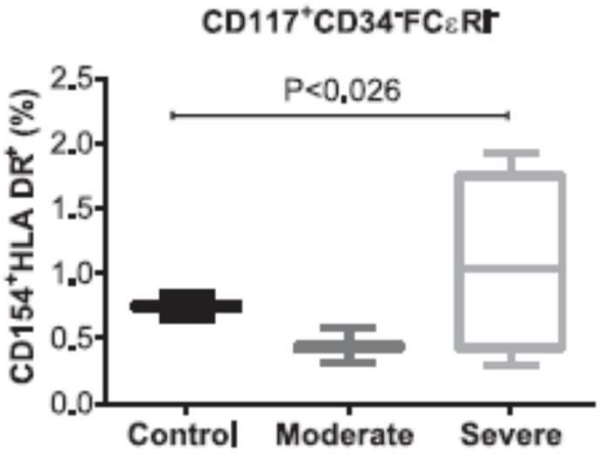
5. Candidates for biomarker validation - immune marker: mast cells

Nguyen *et al*; 2017

Novel characterisation of mast cell phenotypes from PBMCs in CFS/ME patients

- significant increase of naïve MCs in moderate and severe CFS/ME patients (n=18) compared with healthy controls (n=13)
- significant increase in CD40 ligand and MHC-II receptors on differentiated MCs in severe CFS/ME patients



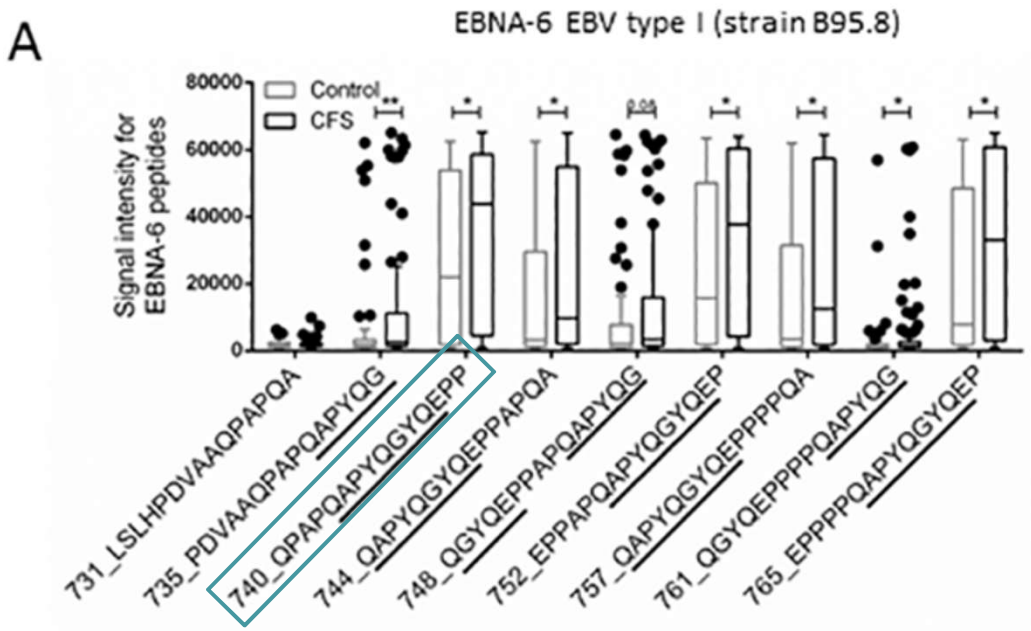


5. Candidates for biomarker validation - immune marker: EBV Serology

Loebel *et al*; 2017

Serological profiling of the EBV immune response in Chronic Fatigue Syndrome using a peptide microarray

- enhanced IgG reactivity against an EBNA-6 repeat sequence



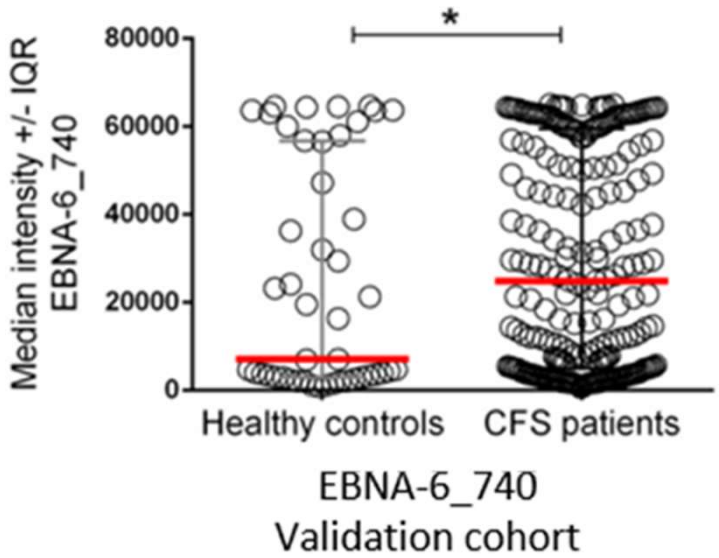
n_{CFS}=93; n_{HD}=40

5. Candidates for biomarker validation - immune marker: EBV Serology

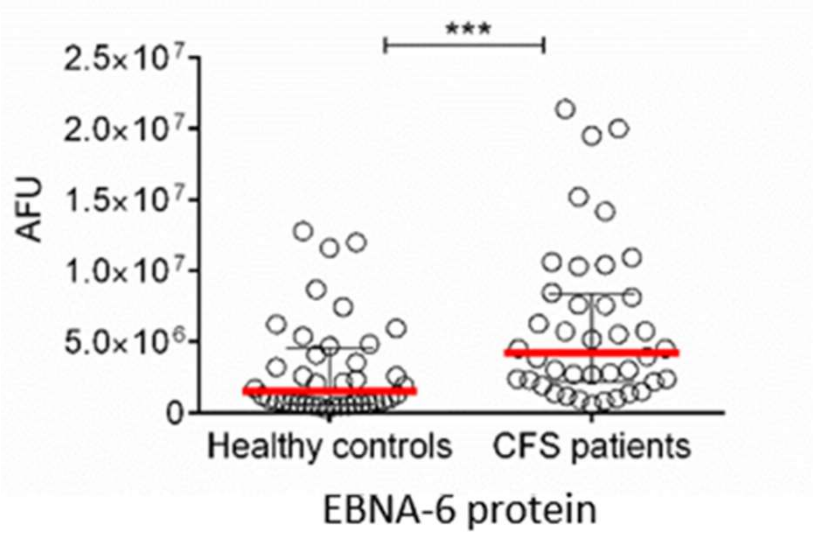
Loebel *et al*; 2017

Serological profiling of the EBV immune response in Chronic Fatigue Syndrome using a peptide microarray

- enhanced IgG reactivity against an EBNA-6 repeat sequence and against EBNA-6 protein is found in CFS patients



n_{CFS}=227; n_{HD}=47



n_{CFS}=40; n_{HD}=40

5. Candidates for biomarker validation
- immune marker: EBV/HHV-6 Serology

Halpin *et al*; 2017

ME/CFS and GWI patients exhibit increased humoral responses to the herpesviruses-encoded dUTPase: Implications in disease pathophysiology

TABLE 3 Grouping of GWI and ME/CFS patients based upon anti-HHV-6, EBV, VZV, and Human dUTPase antibodies[a]

dUTPase Ab subgroup	GWI cohort; *N* (% of total)	ME/CFS cohort; *N* (% of total)	Control cohort; *N* (% of total)
Negative	29 (50)	25 (45.45)	87 (57.6)
HHV-6 only	12 (20.69)	12 (21.82)	20 (13.25)
EBV only	0 (0)	0 (0)	15 (9.93)
VZV only	0 (0)	0 (0)	2 (1.32)
Human only	0 (0)	1 (1.82)	1 (0.67)
HHV-6 + EBV	3 (5.20)	10 (18.18)	13 (8.60)
HHV-6 + VZV	1 (1.72)	0 (0)	2 (1.32)
HHV-6 + Human	2 (3.45)	1 (1.82)	0 (0)
EBV + VZV	0 (0)	0 (0)	1 (0.67)
EBV + Human	0 (0)	0 (0)	0 (0)
VZV + Human	0 (0)	0 (0)	0 (0)
HHV-6 + EBV + VZV	2 (3.45)	0 (0)	4 (2.65)
HHV-6 + EBV + Human	4 (6.90)	4 (7.27)	2 (1.32)
EBV + VZV + Human	0 (0)	0 (0)	0 (0)
HHV-6 + EBV + VZV + Human	5 (8.62)	2 (3.64)	4 (2.65)
Total	58	55	151

29 (52.7%)

45 (29.8%)

8 (14.5 %)

8 (5 %)

n_{CFS}=55; n_{HD}=151

5. Candidates for biomarker validation

- infection marker: HHV-6 & HHV-7 & B19

S. Chapenko, …. M. Murovska, 2006 & **2012**

Viral infection	Plasma samples ($n = 108$)	Practically health persons ($n = 90$)
Active viral infection	70/108	12/90
Single HHV-6	2/108	0/90
Single HHV-7	28/108	10/90
Single B19	11/108	2/90
Dual HHV-6 + HHV-7	10/108	0/90
Dual HHV-7 + B19	15/108	0/90
Triple HHV-6 + HHV-7 + B19	4/108	0/90
Latent viral infection	32/108	53/90
Without viral infection	6/108	25/90

ME/CFS Biomarker Landscape project - potential immune and infection biomarkers

- TGFβ
- serum BAFF
- active HHV-6 & HHV-7/HHV7 & B19 infection
- TRPM3
- Activin B
- mast cells
- EBV serology:
  EBNA6, dUTPase
- sCD26
- BDNF

first validation data available

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