

# **What causes CFS/ME ?**

**Is it all in the Gut ?**

**Research on Extremely Disabled ME Patients Reveals the True Nature of the Disorder**

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# **Myalgic encephalomyelitis:**

## **A highly prevalent debilitating disease**

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- **Persistent, debilitating fatigue associated with numerous physical and neurocognitive symptoms**

Disease severity can range from moderate to extremely severe: patients bedridden for years, totally caregiver dependent

- **Prevalence estimates: 0,3 to 0,6%; one million patients in the USA, two million patients in Europe**

This may just be the tip of the iceberg

- **High socio-economic cost**

Cost to the society estimated as approximately \$16 billion in the USA, €20 billion in Europe

## **Norwegian study: design, parameters tested**

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- **Norwegian study focused on a group of extremely disabled patients**

Purpose of the study: investigate clinical dysfunctions in totally bedridden patients (Karnofski score 20-30) compared to moderately ill patients (Karnofski 60-70), contact controls and non-contact controls

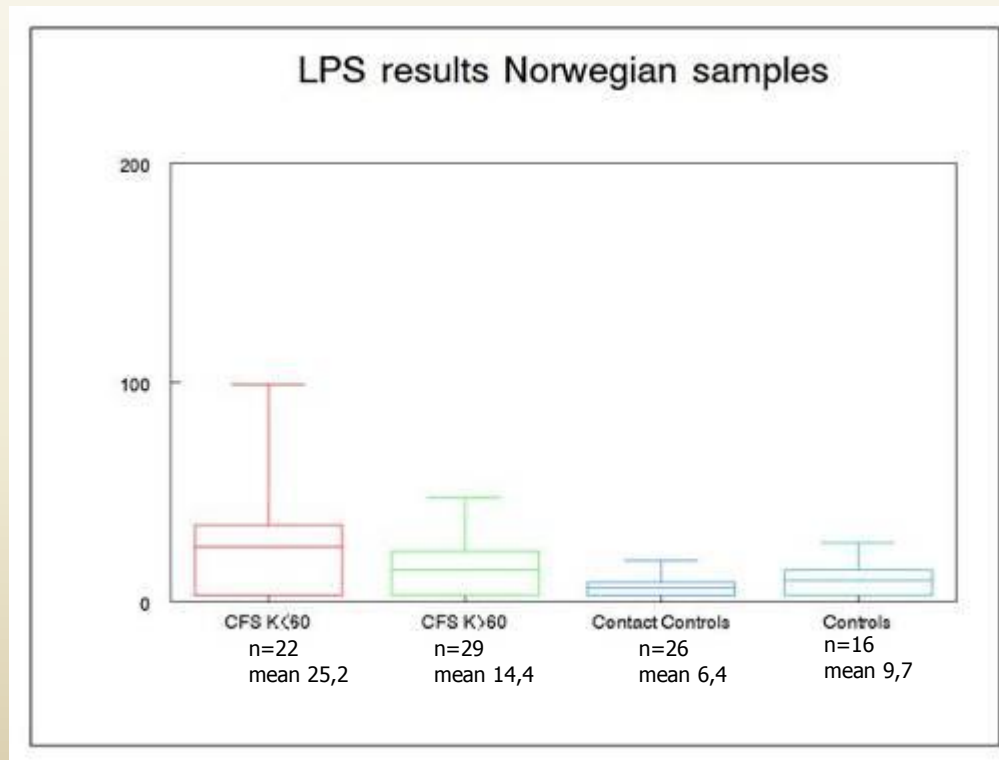
- **Parameters tested included immune function parameters, viruses, intestinal dysfunction markers**

- HHV-6, EBV antibodies: no differences between the groups
- Bornavirus: few positive patients, no more than controls
- HHV-6, EBV PCR: a few positive patients. Not always correlated with antibody titers

## Intestinal dysfunction marker, LPS, was significantly different between the groups

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- Plasma LPS is more elevated in patients with low Karnofski score



## **High levels of plasma LPS are indicative of increased intestinal permeability in ME patients**

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- **LPS is a strong activator of the immune system. Presence of LPS in the bloodstream suggests a hyperpermeable gut, which is consistent with the numerous intestinal symptoms seen in most ME patients**

Nausea

Poor appetite

Gastric reflux

Abdominal pain

Abnormal bowel motility

Bloating

- **Most likely explanation of gut permeability: alteration of the intestinal microbial flora**

## Alterations of intestinal microflora in ME patients (aerobes)

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- *Enterococcus* and *Streptococcus* species are strongly over-represented in ME patients :

Organisms	Control	ME patients	<i>p</i> -value
<i>E.coli</i>	$1.0 \times 10^8$	$4.26 \times 10^7$	$p=0.98$
<i>Enterococcus</i> spp.	$5.0 \times 10^6$	$3.5 \times 10^7$	<b><math>p&lt;0.001</math></b>
<i>Streptococcus</i> spp.	$8.9 \times 10^4$	$9.8 \times 10^7$	<b><math>p&lt;0.001</math></b>

## Alterations of intestinal microflora in ME patients (anaerobes)

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- Among anaerobic bacteria, *Prevotella* is the most consistently overgrown bacteria :

Organisms	Control	ME patients	<i>p</i> -value
<i>Bacteroides</i> spp.	3.2 x 10 <sup>11</sup>	1.6 x 10 <sup>11</sup>	<i>p</i> =0.39
<i>Prevotella</i> spp.	1.0 X 10 <sup>8</sup>	9.0 x 10 <sup>9</sup>	<b><i>p</i>&lt; 0.001</b>
<i>Bifidobacterium</i> spp.	6.0 x 10 <sup>8</sup>	5.5 x 10 <sup>9</sup>	<b><i>p</i>=0.001</b>
<i>Lactobacillus</i> spp.	2.7 x 10 <sup>7</sup>	1.8 x 10 <sup>8</sup>	<b><i>p</i>=0.002</b>

## Alterations of intestinal microflora in ME patients : ratios anaerobes/aerobes and Gm(-)/Gm(+)

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- Gm(-)/Gm(+) ratio are very significantly altered :

Organisms	Control	Patients	<i>p</i> value
Anaerobes / Aerobes	13210.62	11295.19	= 0.08
Gm(-) / Gm(+) ratio	16114.79	658.96	<0.001



# Microbiological assay : sample result

- Patient presents increased *Streptococcus*, *Enterococcus*, and *Prevotella*

**Faecal Microbial Analysis**

Patient ID: 810284 DOB: 31/03/1942 Gender: Female  
Phone (H): (W):  
Name: [REDACTED]  
Address: [REDACTED]

R.I.D. Laboratoria N.V./S.A.  
Z.1 Researchpark 150  
B-1731 Zofe, Belgium  
Phone: +32-(0)2-481-5310  
Fax: +32-(0)2-481-5311

Referred by: Kenny De Meirleir, Physician  
Diagnosis or Comments: [REDACTED]  
Collection Date: 26/04/2022 Receipt Date: 26/04/2022 Entry Date: 26/04/2022 Sample ID: M060226

**Total Counts**

chg/eq	Lo	Hi	Normal Range
Total Bacterial Count	1.4E+10		(1.8E+9 - 1.8E+12)
Aerobe : Anaerobe Ratio	7.8E+00	*	(1.0 - 2.0) = (Aerobe count/Anaerobe count) x 1000

**Aerobic Bacteria**

Lo	Hi	Normal Range
Total Aerobe Count	3.7E+07	(1.0E+7 - 1.0E+8)
<i>Escherichia coli</i>	1.0E+07	(7.8E+6 - 9.0E+7)
<i>Staphylococcus spp.</i>	3.0E+06	<(1.0E+6)
	0.0E+00	<(1.0E+6)
	0.0E+00	<(1.0E+5)
<i>Staphylococcus spp.</i>	0.0E+00	<(2.0E+5)
Total Enterococcus	1.3E+06	<(5.0E+5)
Total Streptococcus	5.0E+07	<(3.0E+5)
alpha-haemolytic streptococcus	3.0E+03	<(3.0E+5)
non-haemolytic streptococcus	3.0E+07	<(3.0E+5)
beta-haemolytic streptococcus	0.0E+00	<(3.0E+5)

**Yeasts**

None isolated	0.0E+00	<(1.0 E+4 (dry))
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**Total Counts**

chg/eq	Lo	Hi	Normal Range
Total Bacterial Count	1.4E+10		(1.8E+9 - 1.8E+12)
Aerobe : Anaerobe Ratio	7.8E+00	*	(1.0 - 2.0) = (Aerobe count/Anaerobe count) x 1000

**Anaerobes**

Total Count	Lo	Hi	Normal range
Total Bacteroides	5.0E+08		(9.0E+7 - 9.5E+11)
<i>Bacteroides fragilis</i> spp.*	5.0E+08		(9.0E+7 - 9.5E+11)
<i>Bacteroides urealyticus</i> spp.*	0.0E+00		0.0%
<i>Prevotella</i> spp.*	0.0E+00	*	<(5.0E+8)
<i>Porphyromonas</i> spp.*	1.5E+08	*	<(5.0E+8)
<i>Eubacterium</i>	5.0E+08	*	<(1.0E+9)
	0.0E+00		<(2.7E+7)
<i>Bifidobacterium</i> *	3.0E+08		(5.0E+6 - 5.0E+8)
	0.0E+00		0.0%
<i>Lactobacillus</i> *	0.0E+00	*	(5.0E+5 - 1.0E+7)
	0.0E+00		0.0%
Total Clostridium	0.0E+00	*	<(3.0E+8)
<i>Clostridium</i> spp.	0.0E+00		0.0%
	0.0E+00		0.0%
	0.0E+00		0.0%
Total Peptostreptococcus	0.0E+00		<(1.0E+5)
<i>Peptostreptococcus</i> spp.	0.0E+00		0.0%
	0.0E+00		0.0%
	0.0E+00		0.0%
	0.0E+00		<(5.0E+4)
	0.0E+00		<(5.0E+4)

# Bacterial overgrowth correlates with symptom severity

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- *Enterococcus* spp. counts correlate with symptom expression :

Symptoms	r and <i>p</i> -values
Headache	r=.17, <i>p</i> <0.01
Arm pain	r=.20, <i>p</i> <0.003
Shoulder pain	r=.15, <i>p</i> <0.04
Myalgia	r=.20, <i>p</i> <0.003
Palpitations	r=.16, <i>p</i> <0.02
Sleep disturbance	r=.20, <i>p</i> <0.004

# Bacterial overgrowth correlates with symptom severity

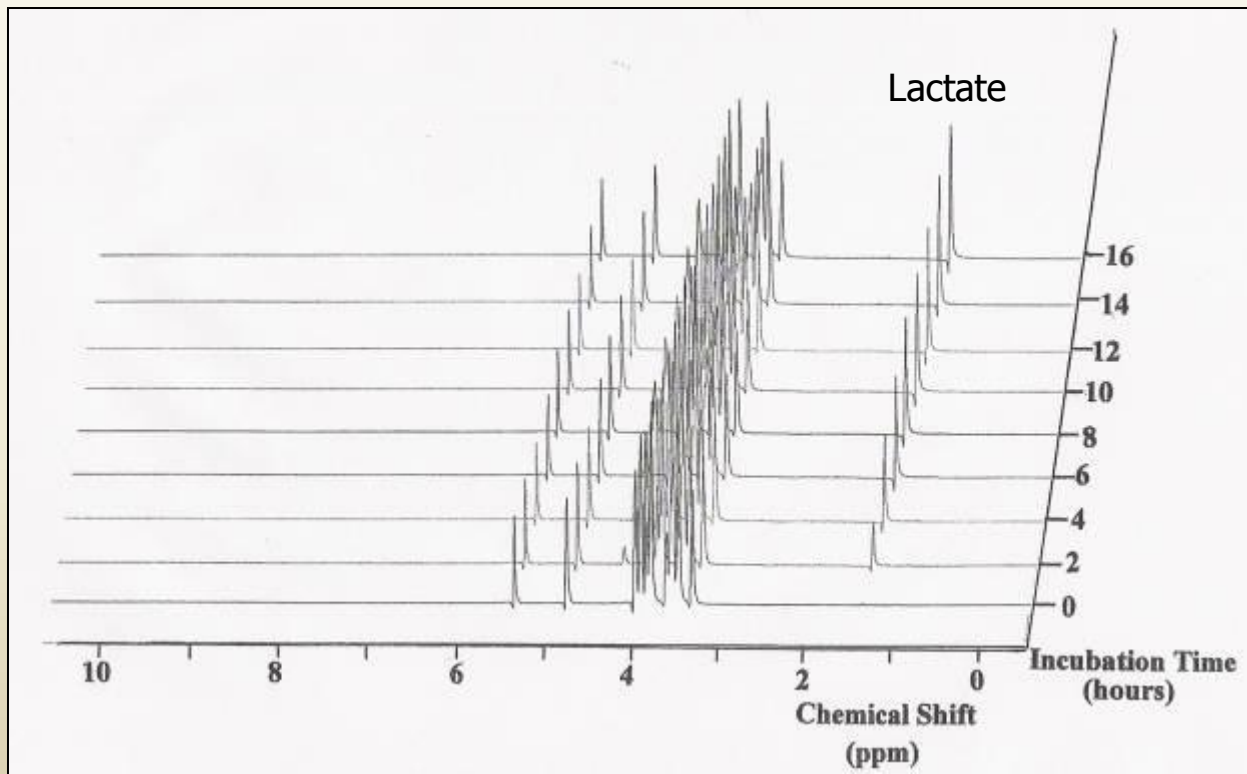
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- *Streptococcus* spp. counts correlate with symptom expression :

Symptoms	r and <i>p</i> -values
Post Exertional fatigue	r=.15, <i>p</i> <0.03
Photophobia	r=.14, <i>p</i> <0.04
Mind going blank	r=.17, <i>p</i> <0.01
Cervical gland lymphodysnia	r=.14 <i>p</i> <0.04
Palpitations	r=.15, <i>p</i> <0.03
Dizziness/Faintness	r=.14, <i>p</i> <0.05

# Overgrown bacteria produce lactic acid

- NMR metabolic profiles of *Enterococcus faecalis*



## Increased production of lactic acid (Lactic Acidosis) could contribute to symptoms

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- “Lactic acid” bacteria counts correlate with symptom expression :

Symptoms	$r$ , $p$ -values
Mental fatigue	$r=.18$ , $p<0.009$
Photophobia	$r=.18$ , $p<0.008$
Urinary frequency	$r=.16$ , $p<0.03$
Urinary urgency	$r=.14$ , $p<0.04$
Palpitations	$r=.15$ , $p<0.03$
Dizziness/Faintness	$r=.13$ , $p<0.05$

## Another potential toxin produced by bacteria is hydrogen sulfide (H<sub>2</sub>S)

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- **Hydrogen sulfide (H<sub>2</sub>S) has important physiological functions...**

H<sub>2</sub>S is produced by the cells and is an important gaseous signal molecule, involved in regulation of blood pressure, neurotransmission, muscle relaxation and regulation of inflammation

- **...but exogenous exposure can be extremely toxic**

In excess, H<sub>2</sub>S acts as a mitochondrial poison. It can directly inhibit enzymes involved in the cellular production of energy. H<sub>2</sub>S also interferes with oxygen transport by blocking hemoglobin in the red blood cells. H<sub>2</sub>S is a potent neurotoxin

***Enterococcus, Streptococcus, Prevotella* are strong H<sub>2</sub>S producers**

# A marker associated with H<sub>2</sub>S production can be measured with a simple urine test

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**1. Collect urine**



**2. Open tube containing test reagent**



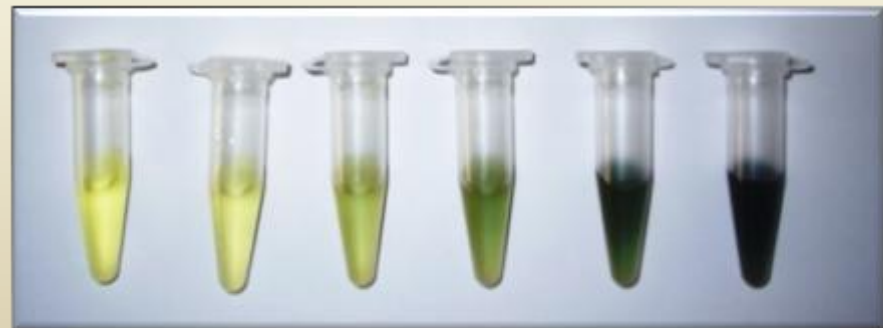
**3. Add a few drops of urine to the test reagent**



**4. Mix by shaking gently.  
Wait for two minutes**



**5. Observe color changes. Dark color = positive sample**




Negative or  
Pre-ME

Moderate  
disease

Severe  
disease

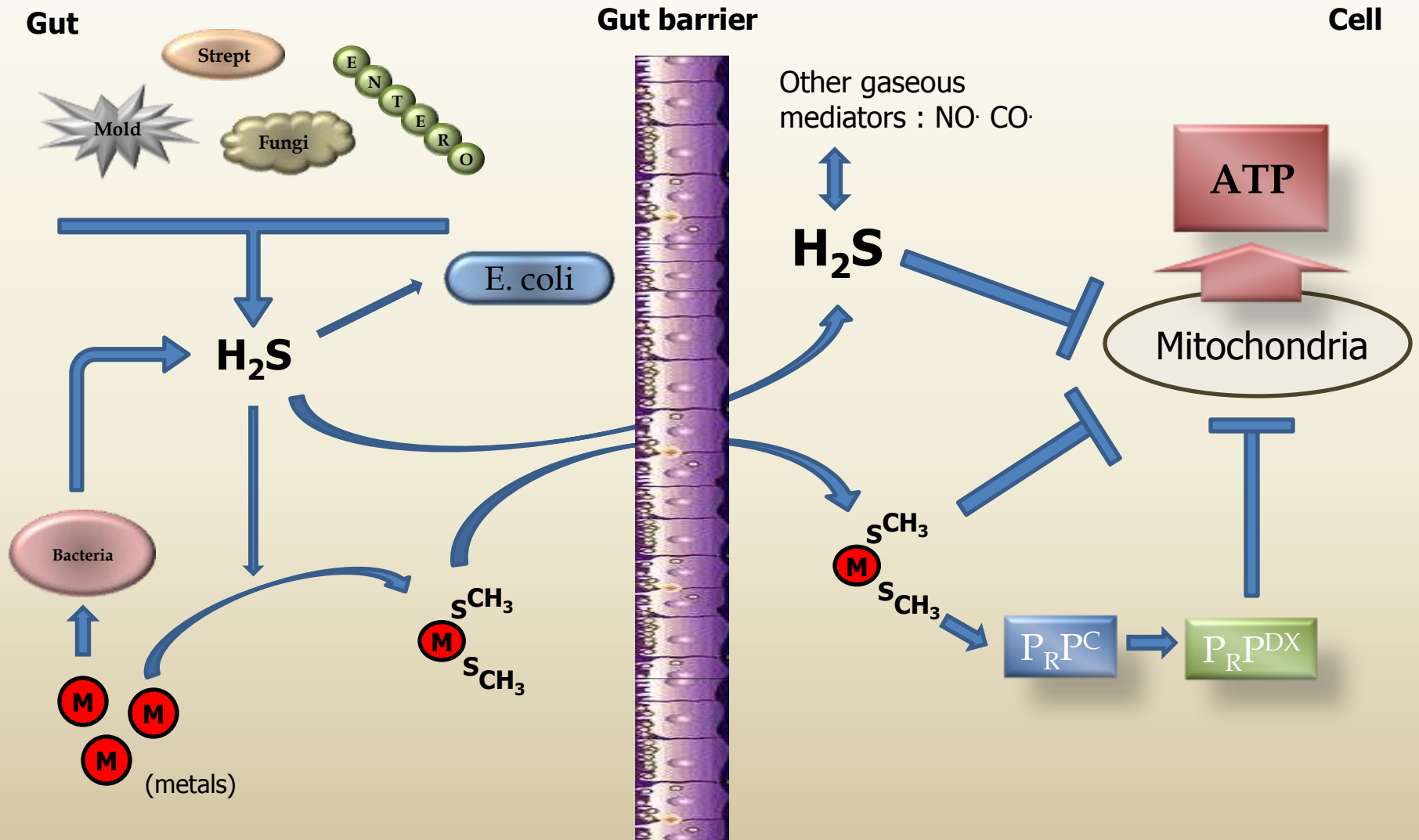
# Heavy metal exposure is another contributing factor to the disease

- Patients often present intoxication with mercury, nickel or other metals

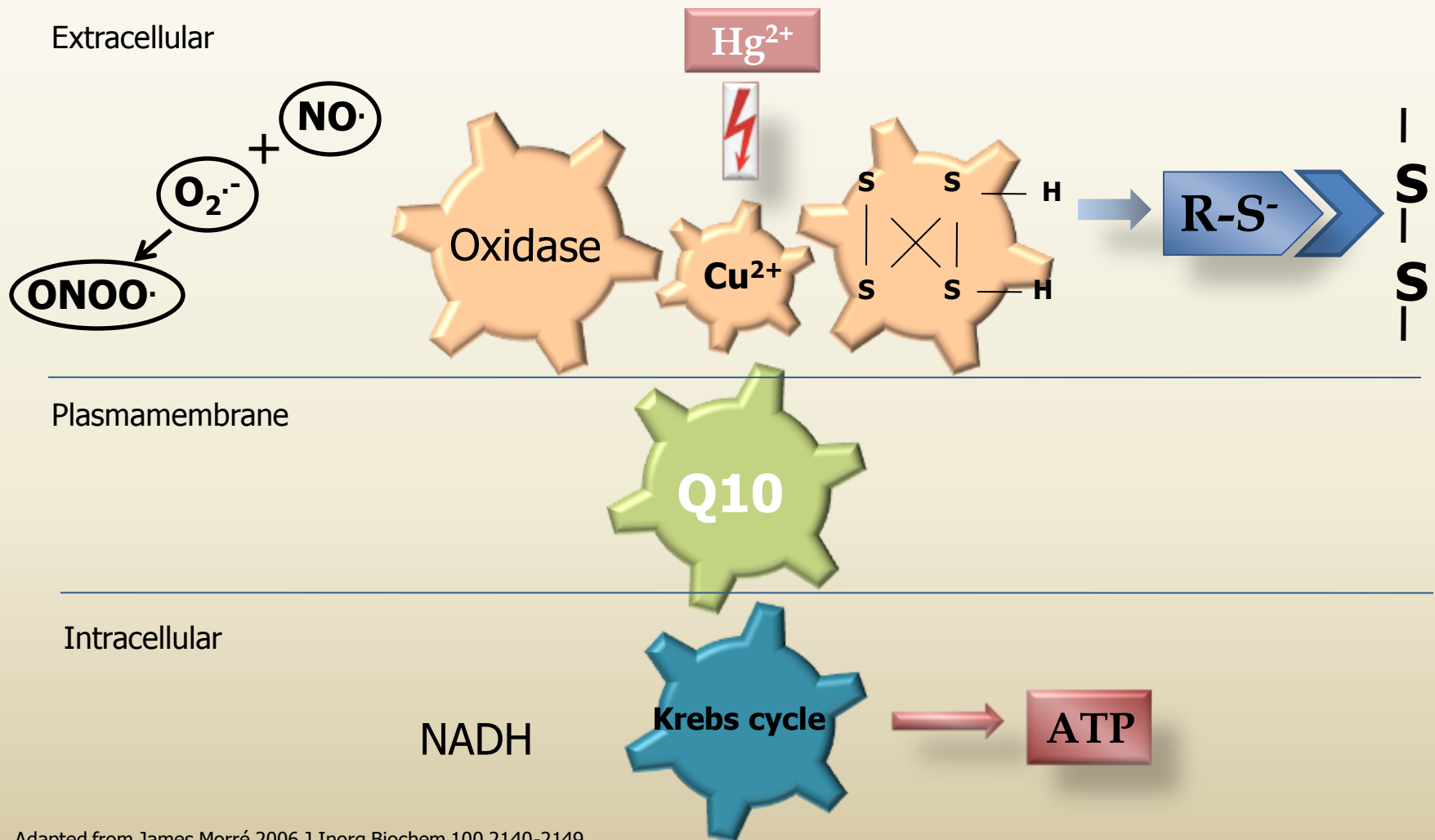
<b>MICRO TRACE MINERALS GmbH</b> environmental & clinical laboratory			
Röhrenstrasse 20 D-91217 Hainbrunn USA, P.O. Box 4813, Boulder, Co 80306-4813		Telefon: +49 (0) 91514332 Telefax: +49 (0) 91512306 <a href="http://www.microtrace.de">http://www.microtrace.de</a> <a href="mailto:service@microtrace.de">service@microtrace.de</a>	
<b>MINERAL ANALYSIS</b>		<b>Urine</b>	<b>Lab Number</b> EUR92853
Doctor	Prof. Dr. K. De Meirle		
Patient Name			
Clinical Information	Dose: 250mg, Sol. DMPS+150ml NaCl 0.3% 2hr		
Test Date	20. Mai. 09	D.O.B.	31.12.1987
		Sex	F
		Creatinine (g/l)	0.3
Essential Macro- & Trace Elements (mg/g creatinine)		Low	Acceptable Range High
Acceptable Range Test Value			
Calcium	55.00 – 245.00	137.05	*****
Magnesium	12.00 – 150.00	89.75	*****
Zinc	0.07 – 7.00	3.64	*****
Essential Trace Elements (mcg/g Creatinine)		Low	Acceptable Range High
Acceptable Range Test Value			
Chromium	0.10 – 3.50	0.00 Low	<
Cobalt	< 5.00	0.97	*****
Copper	1.45 – 60.00	651.86 High	*****
Iron	2.00 – 95.00	12.34	*****
Manganese	< 4.50	2.95	*****
Molybdenum	9.70 – 100.00	13.03	*****
Selenium	12.00 – 90.00	10.24 Low	*****
Vanadium	< 70.00	0.24	*****
Potentially Toxic Elements in mcg/g Creatinine		Low	Acceptable Range High
Acceptable Range Test Value			
Aluminium	< 125.00	19.75	*****
Arsenic	< 15.00	7.67	*****
Barium	< 8.22	1.04	*****
Beryllium	< 1.20	0.94	*****
Cadmium	< 1.50	0.13	<
Lead	< 5.00	4.88	*****
Mercury	< 1.00	16.56 High	*****
Nickel	< 3.00	27.69 High	*****
Silver	< 1.40	1.47 High	*****
Tin	< 5.00	3.23	*****
* The 95percentile Ranges represent baseline urine values and are calculated on the creatinine value. The utilized range is 0.3 to 3.0 g/L creatinine (WHO 2005). For chelator-specific information see attachments.			
Accreditation: DIN EN ISO 17025; Quality control: Dr. Rauland PhD; Validation: Dr. E. Blumrock-Busch PhD			



# H<sub>2</sub>S and heavy metals have cumulative effects, causing reduction of ATP production

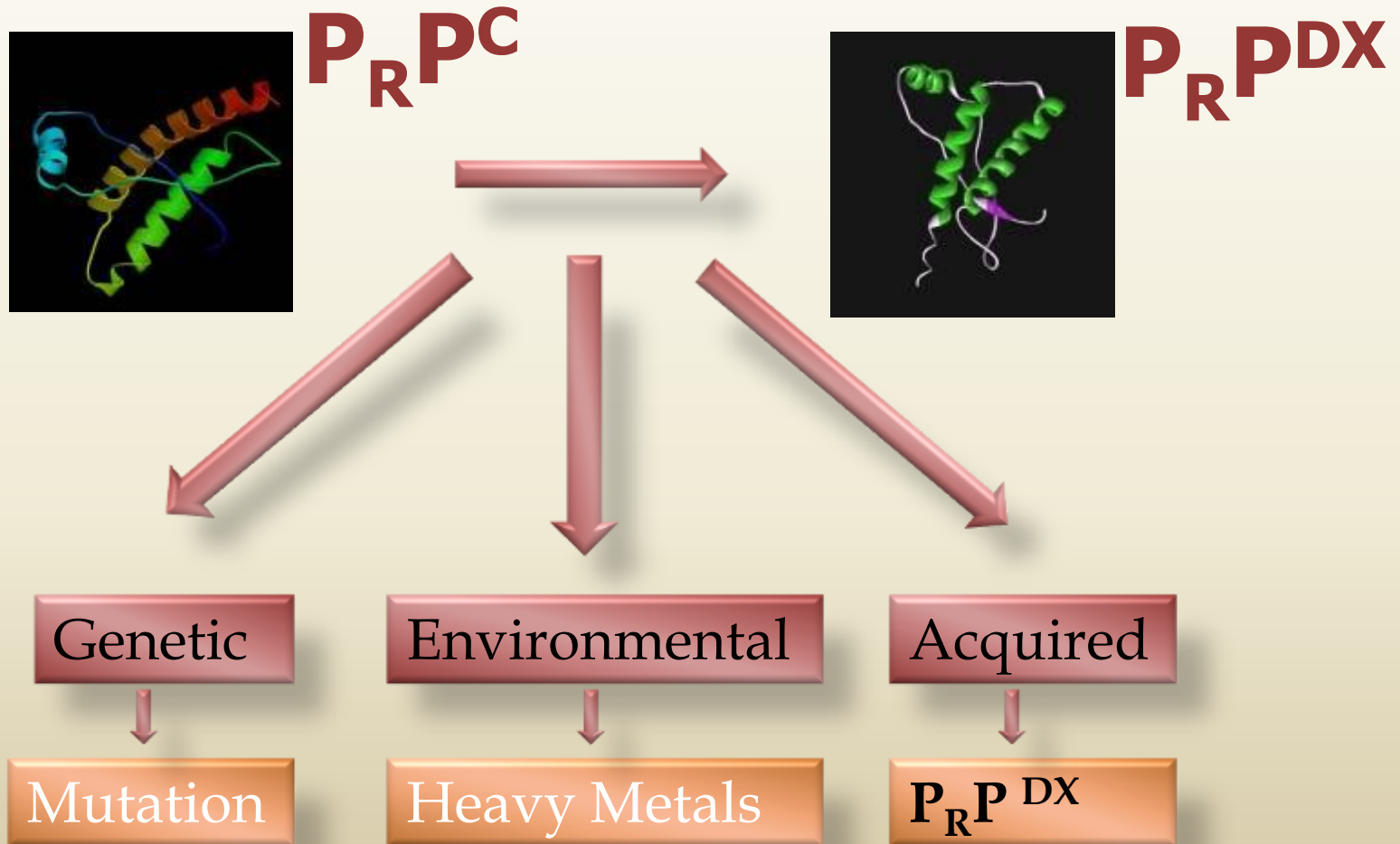


# Heavy metals interfere directly with energy production



# Genetic and environmental factors contribute to aberrant protein conformation

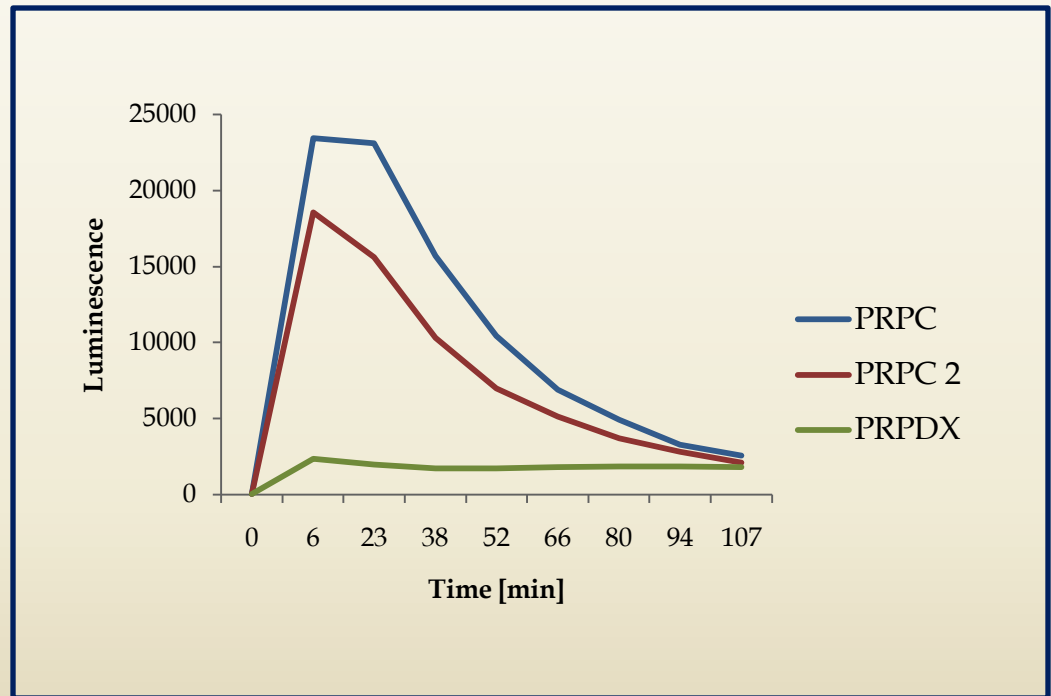
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# Abnormal protein conformation assay

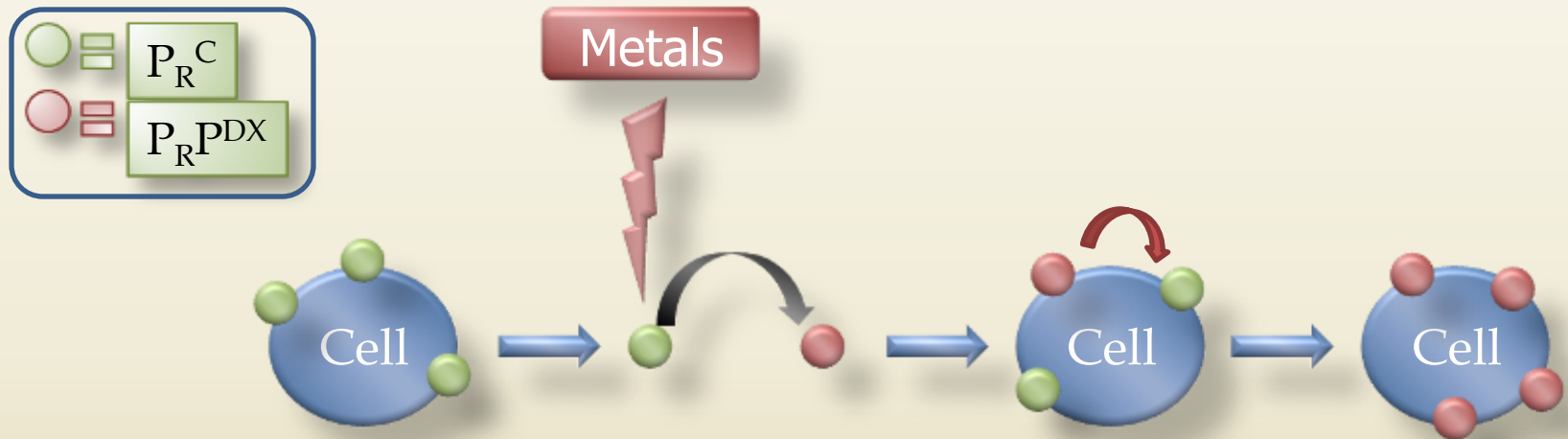
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- **Aberrant luminescence response indicates abnormal conformation**



# Abnormal conformation can be transmitted from one cell to another

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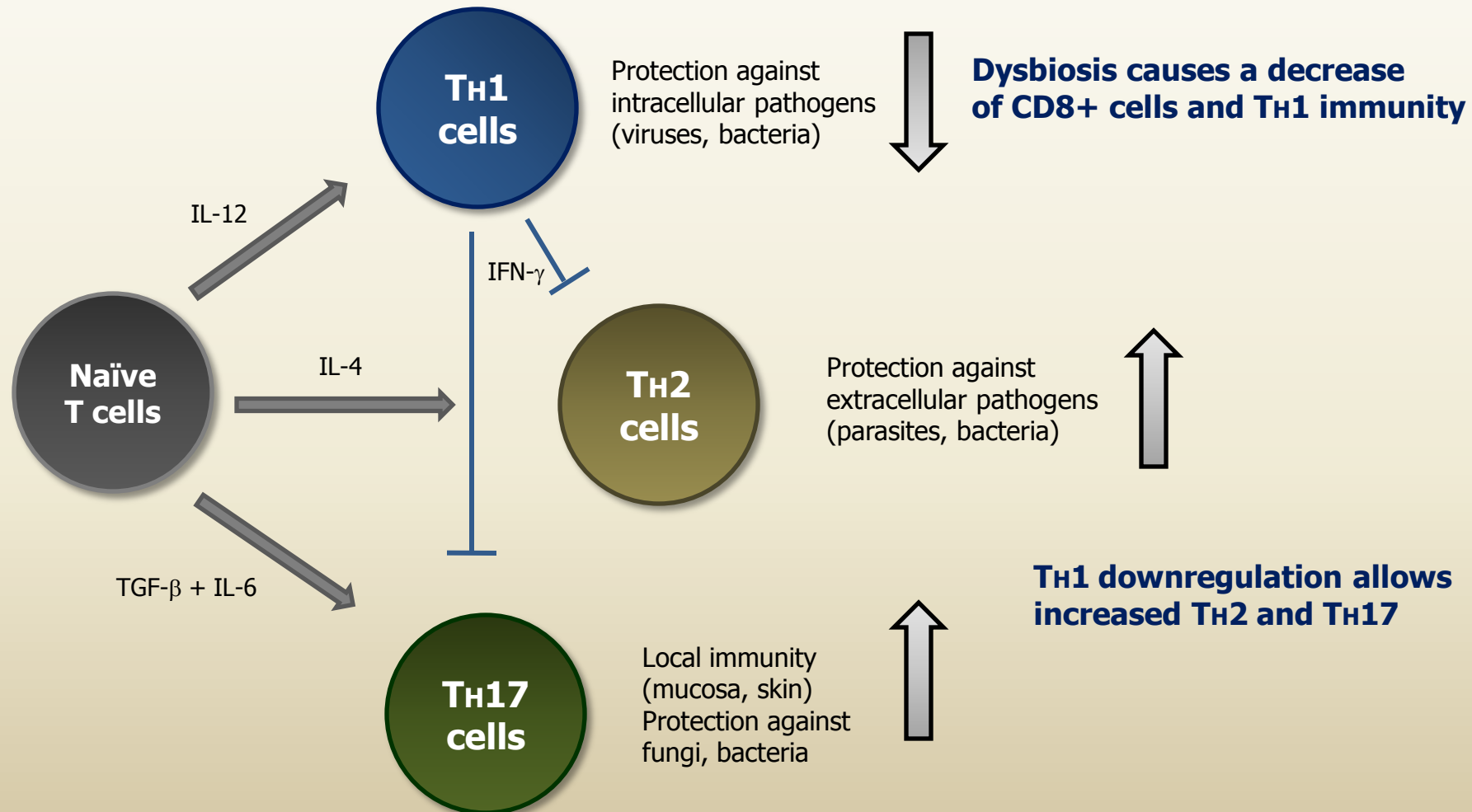
# Disease severity in ME is associated with different physiological dysfunctions

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	<b>I</b> <b>"Pre-ME"</b>	<b>II</b> <b>Moderate disease</b>	<b>III</b> <b>Severe disease</b>
<b>Dysfunctions</b>	Abnormal faecal test, high H <sub>2</sub> S	Abnormal faecal test, high H <sub>2</sub> S, exposure to heavy metals	Abnormal faecal test, high H <sub>2</sub> S, exposure to heavy metals that has caused aberrant protein conformation (APD)
<b>Symptoms</b>	No fatigue, possible gastro-intestinal symptoms. Low VO <sub>2</sub> , slow recovery. May be asymptomatic	Fatigue, gastro-intestinal symptoms	Strong fatigue, multiple symptoms
<b>Treatment</b>	Restore the gut: probiotics	Restore the gut: probiotics, enterocoated antibiotics. Metal chelation, Zinc supplementation	Difficult. Gut restoration, metal chelation. Treatment of associated dysfunctions (opportunistic infections). Treatment of APD is still experimental

Increasing immune dysregulations (depressed T and NK cells, Th17 activation, opportunistic infections...)

# Immune alterations resulting from intestinal dysfunction



# Consequences of altered immunity

- **TH1 decrease favors the development of opportunistic viral infections**

HHV-6, Epstein-Barr, parvovirus B19, enteroviruses are found in ME patients. Gastro-intestinal mucosa is a major site of infection

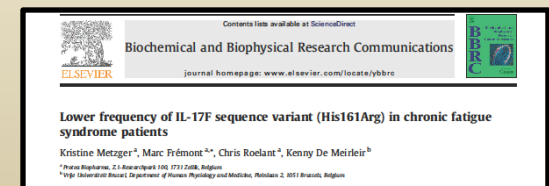


- **TH2 increase favors the development of allergies**

- **TH17 increase promotes inflammation, autoimmunity, blood-brain barrier disruption**

## Genetic background plays a role in T<sub>H</sub>17 upregulation

Polymorphisms of IL-17F, IL-6, TLR4, TGF- $\beta$  genes are associated with ME and other intestinal diseases (Crohn's disease, UC, IBS)





# The case of the XMRV virus

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- **XMRV is an infectious gamma retrovirus**
  - “Xenotropic murine leukemia virus-related virus”
  - Causes mild immuno-suppression
  - First isolated in patients suffering from a form of prostate cancer associated with a defective RNase L
- **An association between XMRV infection and CFS has been reported**
  - In a recent report, found in the blood cells of 67% of CFS patients (68/101) vs. 3.7% of controls (8/218)
  - more research needed to know whether XMRV is a causal factor in the pathogenesis of CFS or reactivates as a consequence of depressed immunity in already sick people
  - Like for other retroviruses, co-infections with other viruses (HHV-6) may be an important issue

## **Patient evaluation**

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- **Urine test for marker associated with H<sub>2</sub>S production**
- **Intestinal microflora evaluation**
- **Heavy metals analysis**
- **Presence of proteins with abnormal conformation**
- **Assays evaluating subsequent immune dysfunctions (immune dysregulations, opportunistic infections...)**

# CONCLUSIONS

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- **Gastro-intestinal dysfunctions play a central role in the pathogenesis of ME**
- **Dysbiosis detrimental effect mediated by increased production of H<sub>2</sub>S**
- **Immune dysfunctions and opportunistic infections arise as a consequence of pre-existing intestinal problems**

Once established, infections will contribute to the maintenance/aggravation of the disease

# Acknowledgements

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- **Henry Butt at the Bio21 Institute, University of Melbourne**



- **Marian Dix Lemle, Independent Researcher, Washington DC**

*Med Hypotheses. 2009 Jan;72(1):108-9. Epub 2008 Sep 16. Hypothesis: chronic fatigue syndrome is caused by dysregulation of hydrogen sulfide metabolism. Lemle MD.*