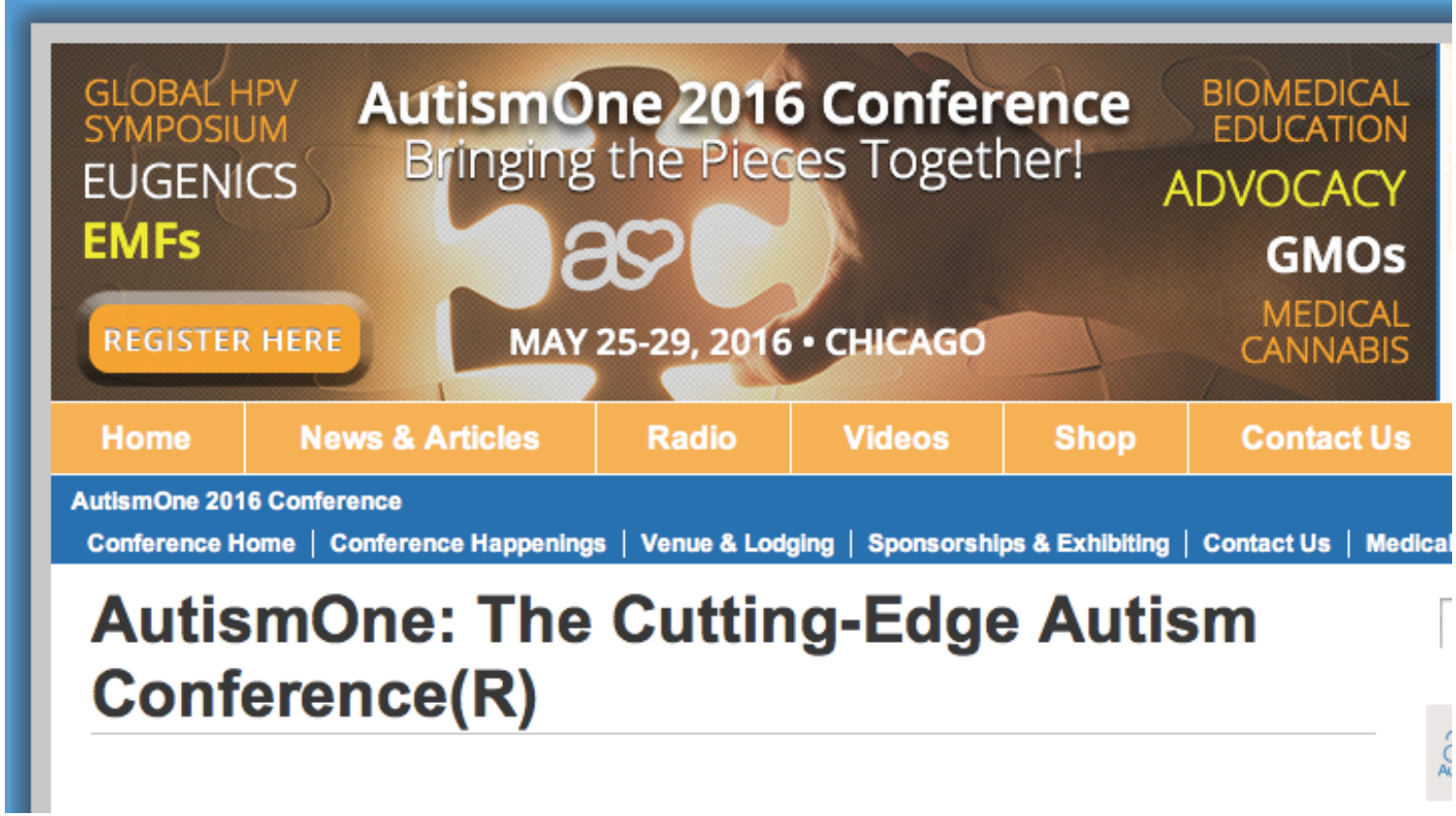


Novel insights on the aetiology and treatment of autism.

Marco Ruggiero, MD, PhD

May 27, 2016

Declaration of conflict of interest

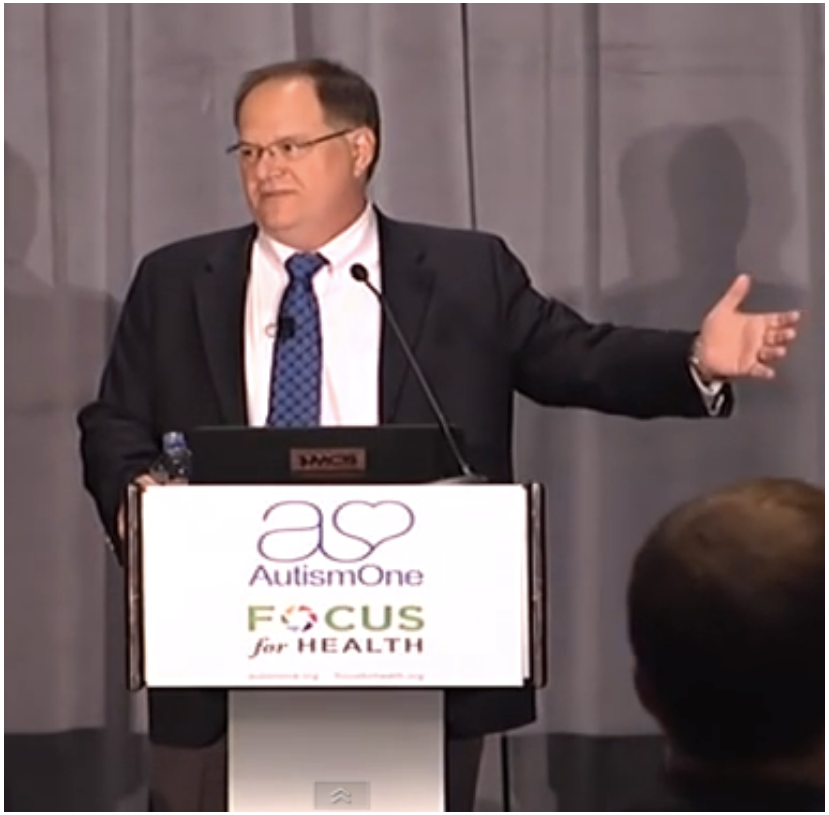
- Marco Ruggiero is the inventor, together with his wife and lifelong scientific collaborator, Dr. Stefania Pacini, of the **Bravo®** yogurt and its derivatives, and they hold shares in the companies producing and distributing Bravo.

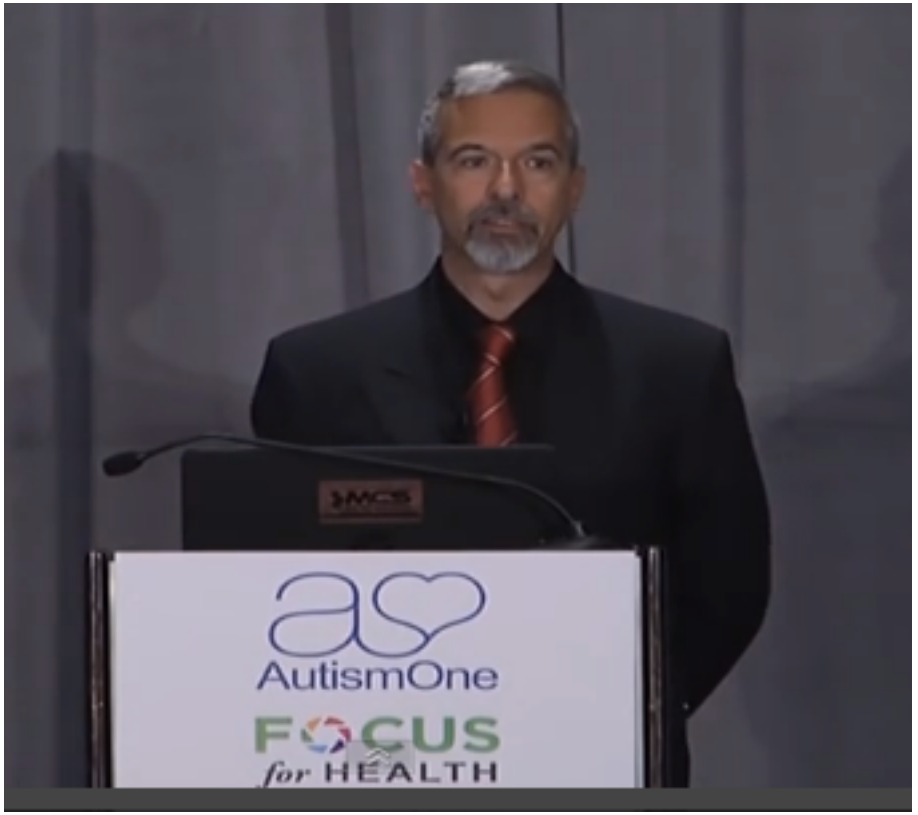
- Marco Ruggiero is the inventor of **Rerum®**, the **evolution** of the GcMAF.

- **Please notice**: the following are academic and scientific considerations and **not medical prescriptions or advices**.
- **Please notice**: the results reported in this talk have been observed in **different countries** in the world. Please check with your National Health Authorities for the current legal situation concerning the approaches described in this talk.
- Please notice: **none of the information that will be presented is to be interpreted as a "cure" for autism or any other disease**.

This presentation has a long history that dates back to

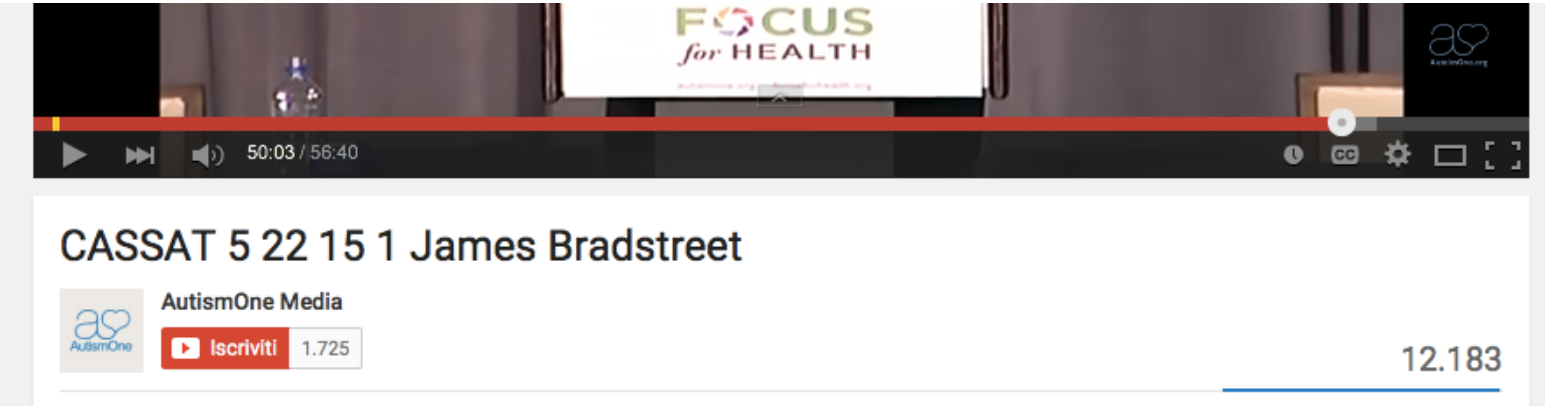
the **2015** AutismOne Conference in Chicago.

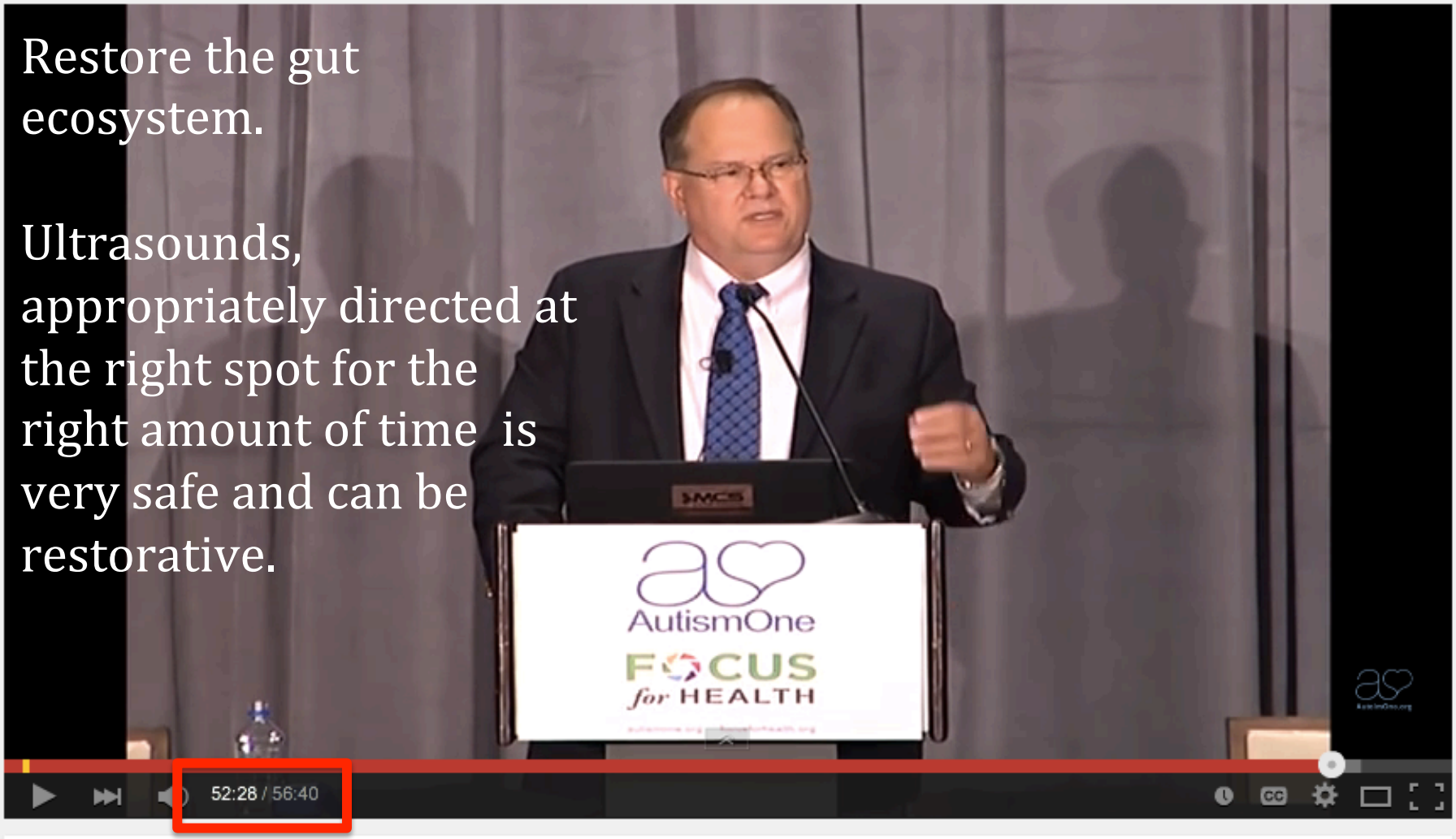




- It is with extreme sadness that today I have the honour to report the results of the research work that Jeff and I had begun more than one year ago.

- I shall begin by quoting the last public words by Dr. Bradstreet pronounced at around min 50 of his last talk at AutismOne in 2015.



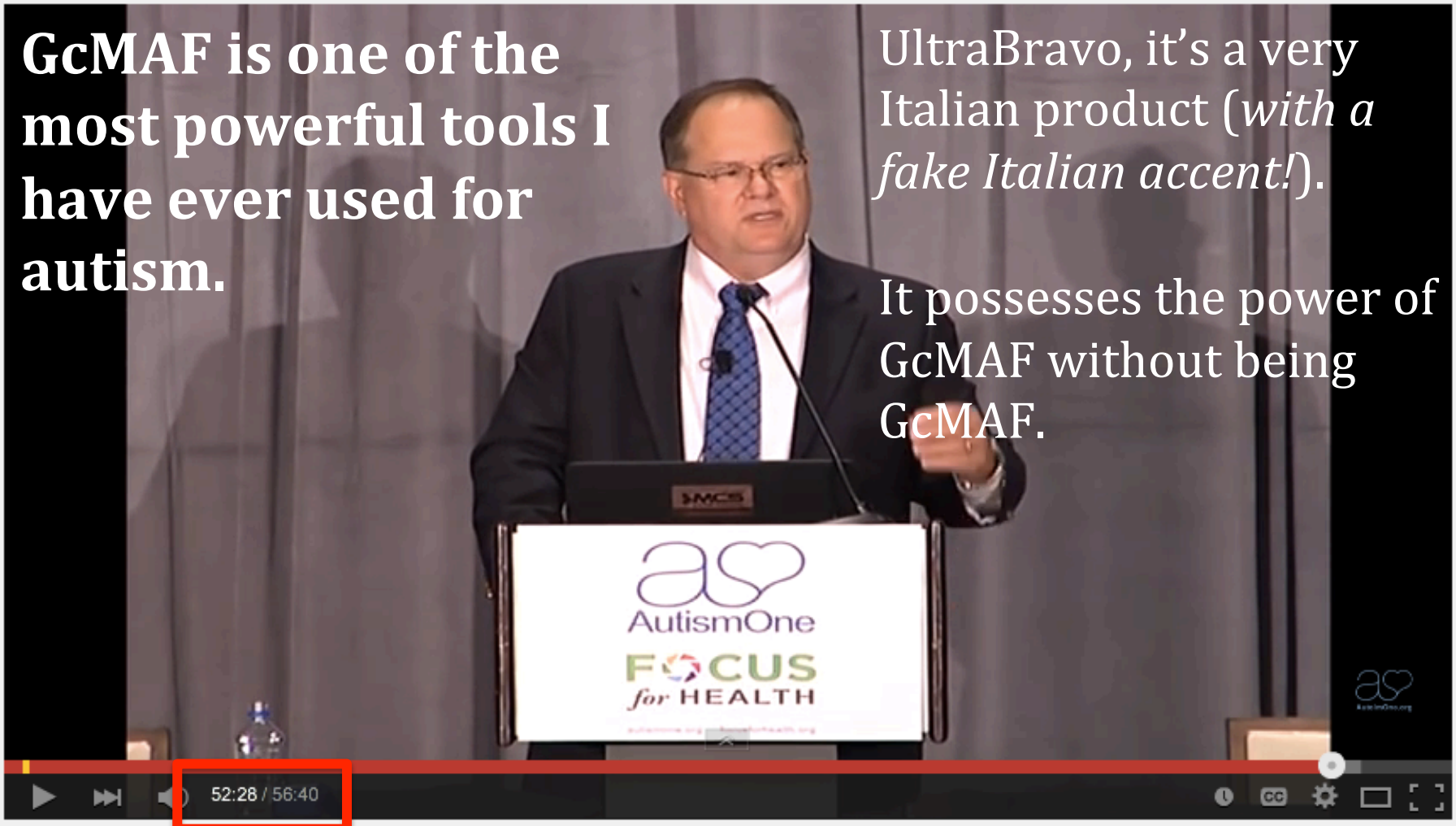


CASSAT 5 22 15 1 James Bradstreet

AutismOne Media

Iscriviti 1.719

12.129



CASSAT 5 22 15 1 James Bradstreet

AutismOne Media

Iscriviti 1.719

12.129

Re: UltraBravo effects

Marco Ruggiero <marco.drruggiero@gmail.com>
to James

6/9/15

It truly does. Thank you so much.

Marco

2015-06-09 18:03 GMT-07:00 James Bradstreet <drbradstreet@gmail.com>:
> This may brighten your evening
>
> ---------- Forwarded message ----------
> From: **luana**
> Date: Tue, Jun 9, 2015 at 8:59 PM
> Subject: UltraBravo effects
> To: James Bradstreet <drbradstreet@gmail.com>
>
> Hi doc! I can't wait to tell you that after about 30 min after taking UltraBravo, Matteo becomes playful, happy, funny and mouthy! He had the first 30 min of fatigue and after makes me laugh all time! :)
> I'm sad because my English is really bad and I can't tell you everything I want about your kindness! I know you are my hero because you gave me back Matteo, but I discovered you are also a great man...
> Thank you so much
> Have a good night
> Luana
>
> Inviato da iPhone

Congratultions Inbox x

James Bradstreet <drbradstreet@gmail.com> 6/17/15

to me, stefania.pacini

I am very proud and impressed with your accomplishments and congratulate you both and your team on the acceptance of the ultrasound effects paper. Bravo or better said UltraBravo.

James Jeffrey Bradstreet, MD, FAAFP, FMAPS
Brain Treatment Center
4488 Commerce Dr. Suite B

Biomedical Signal Processing and Control 22 (2015) 44–53

Contents lists available at ScienceDirect

Biomedical Signal Processing and Control

journal homepage: www.elsevier.com/locate/bspc

Effect of ultrasounds on neurons and microglia: Cell viability and automatic analysis of cell morphology

Leonardo Bocchi[a], Jacopo J.V. Branca[b], Stefania Pacini[b,*], Angela Cosentino[a], Gabriele Morucci[b], Marco Ruggiero[c]

In those days of 2015,

- Jeff Bradstreet and myself had decided to merge the protocols that we had developed thus far, and to perform advanced research to **elucidate the causes** of autism and to establish a protocol **targeting** those causes.

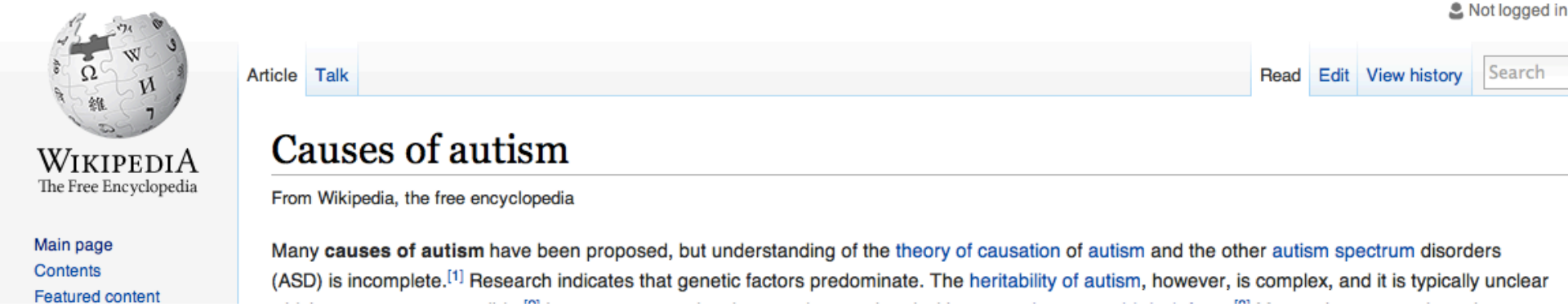
AutismOne 2015 Conference

Conference Home | Conference Happenings | Venue & Lodging | Sponsorships & Exhibiting | Childcare

The Swiss Protocol for autism: an integrated protocol based on nutrition, immunity, and reconstitution of the healthy microbiome.

ESSENCE (Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations)

- In order to be able to device a successful therapeutic protocol, the **aetiology and the pathogenesis** of a diseases have to be known, possibly at the **molecular level**.



A few days before June 19, 2015

- We submitted a paper to the prestigious scientific journal “Frontiers in Neuroscience” a journal of the Nature Publishing Group where we had published a paper describing the lesions in the brain of autistic children one year earlier.

frontiers in
HUMAN NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 15 January 2014
doi: 10.3389/fnhum.2013.00934

A new methodology of viewing extra-axial fluid and cortical abnormalities in children with autism via transcranial ultrasonography

James Jeffrey Bradstreet[1,2,3]*, Stefania Pacini[4] and Marco Ruggiero[5,6]

[1] Newport Brain Research Laboratory, Newport Beach, CA, USA
[2] Brain Treatment Center, Newport Beach, CA, USA
[3] Brain Treatment Center, Buford, GA, USA
[4] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
[5] Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy
[6] Immuno Biotech Ltd., Saint Peter Port, Guernsey

frontiers in Neuroscience | Child and Adolescent Psychiatry

GENERAL COMMENTARY ARTICLE

Front. Neurosci., 22 December 2015 | http://dx.doi.org/10.3389/fnins.2015.00485

Commentary: Structural and functional features of central nervous system lymphatic vessels

 James J. Bradstreet[1,2],  Marco Ruggiero[3*] and  Stefania Pacini[4]

[1]The Brain Treatment Center, Buford, GA, USA
[2]Faculty for Autism Collaboration & Education, Western University of Health Sciences, Pomona, CA, USA
[3]Dream Master Laboratory, Gilbert, AZ, USA
[4]Department of Experimental and Clinical Medicine, University of Florence, Firenze, Italy

A commentary on
Structural and functional features of central nervous system lymphatic vessels

by Louveau, A., Smirnov, I., Keyes, T. J., Eccles, J. D., Rouhani, S. J., Peske, J. D., et al. (2015). Nature 523, 337–341. doi: 10.1038/nature14432

Autism spectrum disorders (ASD) represent an apparent pandemic threat to child development with the current CDC data documenting ASD affecting over 2% of U.S. males of school age ([CDC] Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal, 2014). ASD are likely a heterogeneous group of disorders with genetic and environmental causes resulting in similar phenotypes. Genetic contributions to autism are extremely heterogeneous and may involve synaptic formation and maturation. Thus, multiple genes involved in the formation, specification, and maintenance of synapses have been identified as risk factors for ASD development (Hahn et al., 2013). Also the rate of brain growth in the first 2 years of life may contribute to ASD. Although abnormally enlarged brain volumes and increased rates of brain growth during early childhood are observed only in a minority of ASD children, nevertheless there is evidence of abnormalities in

In this paper

- We put forward a hypothesis that explains the lesions in the brain of autistic children and how to address them.

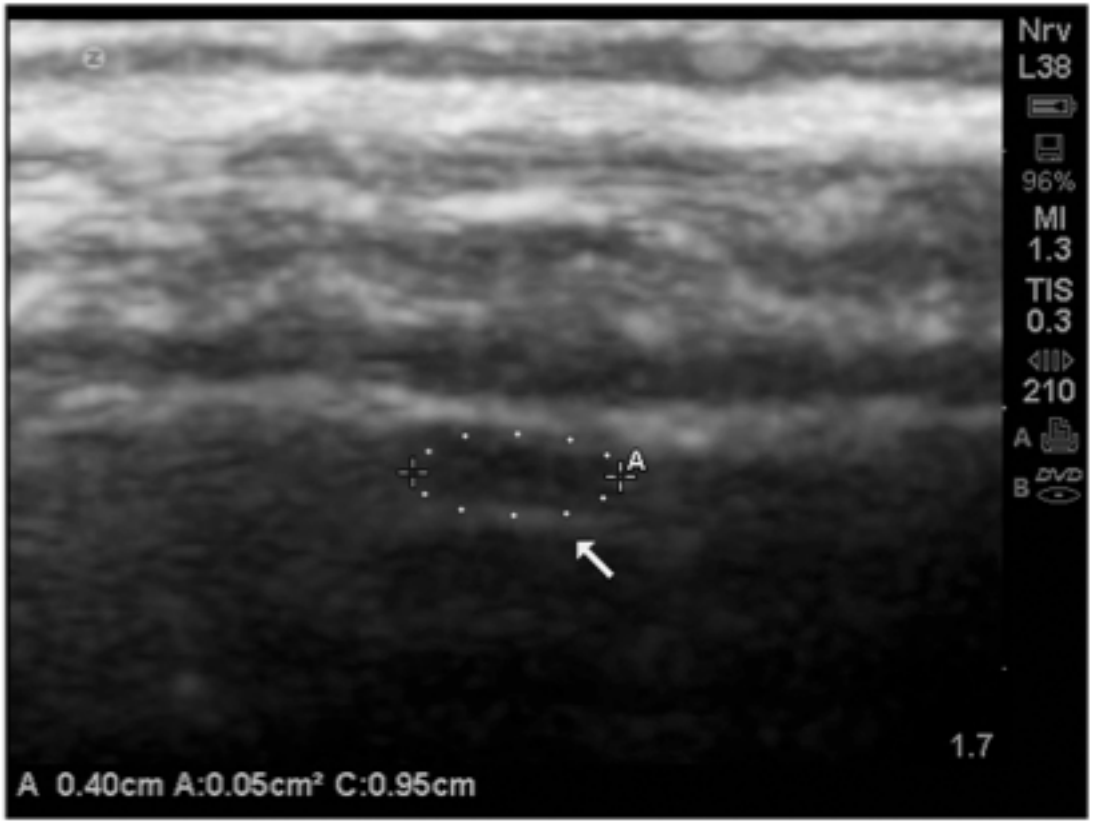


FIGURE 9 | Isolate cortical lesion compatible with a grade 3 lesion due to its size. SonoSite zoom magnification. The arrow points to the enhancement distal to the hypoechoic area which indicates this is unlikely

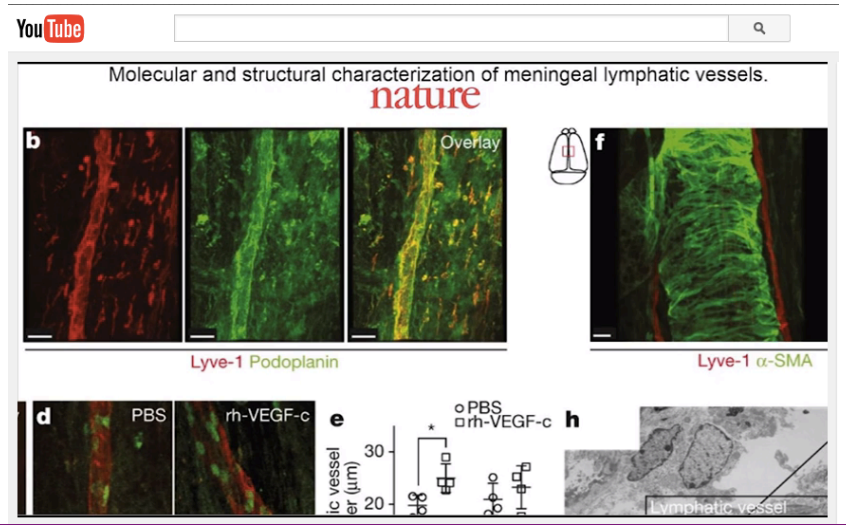
Our hypothesis is based on the revolutionary findings published by Louveau *et al*. from the University of Virginia, USA.

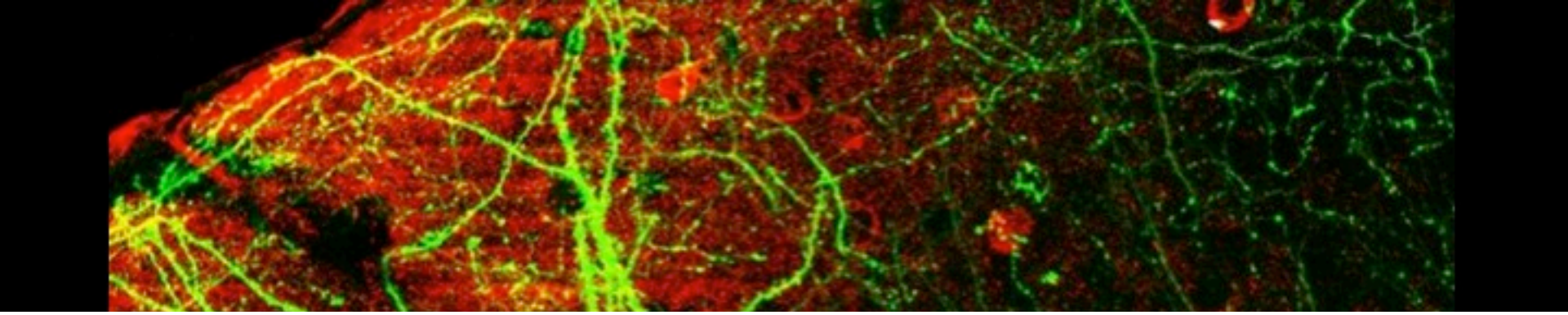
LETTER

doi:10.1038/nature14432

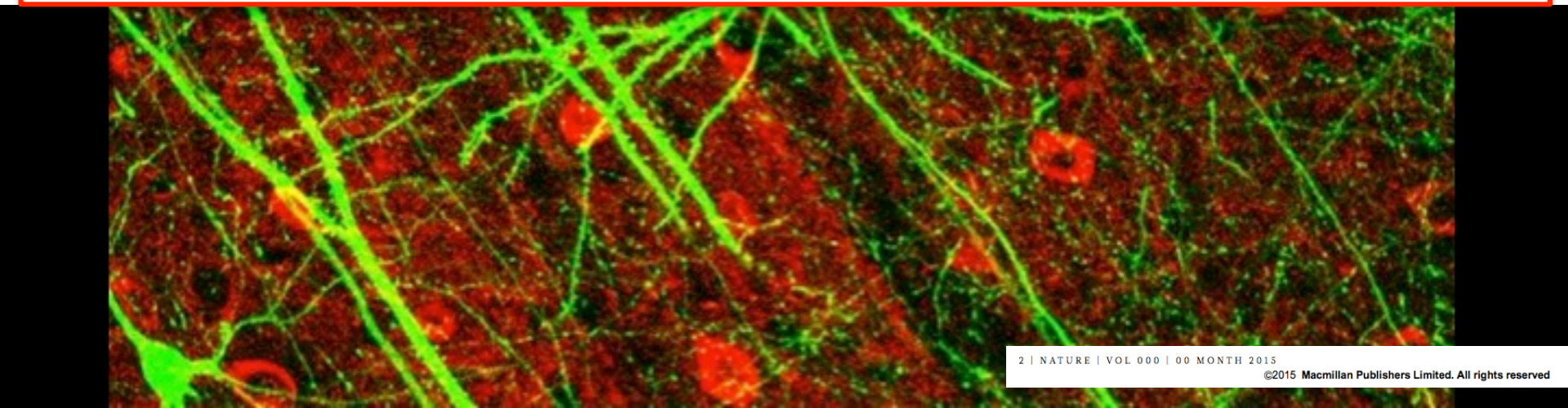
Structural and functional features of central nervous system lymphatic vessels

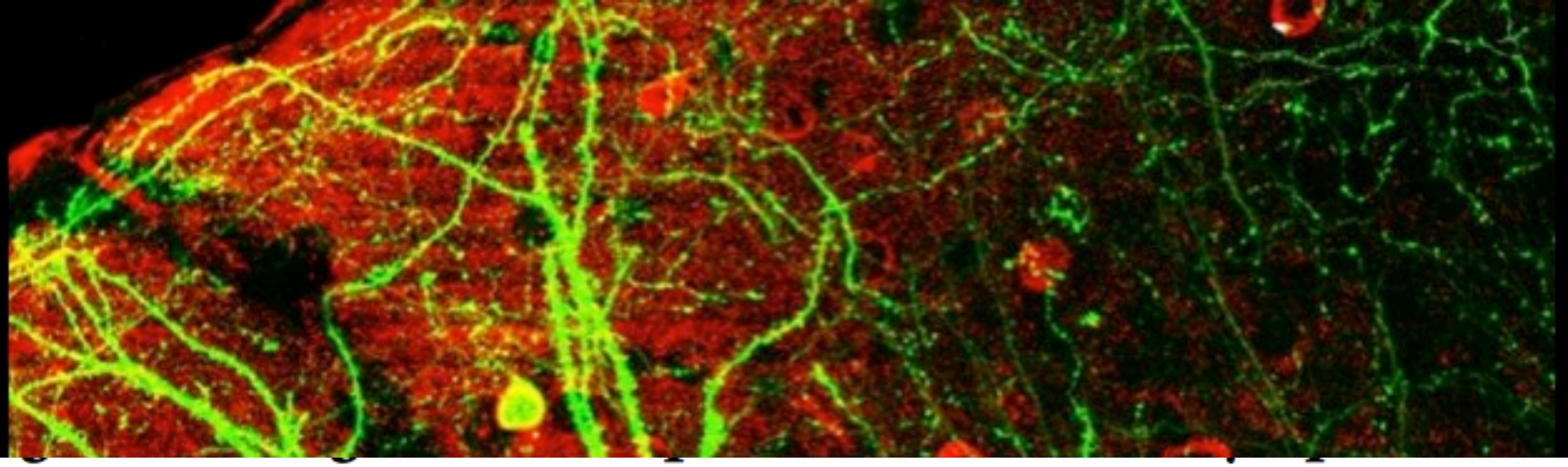
Antoine Louveau[1,2], Igor Smirnov[1,2], Timothy J. Keyes[1,2], Jacob D. Eccles[3,4,5], Sherin J. Rouhani[3,4,6], J. David Peske[3,4,6], Noel C. Derecki[1,2], David Castle[7], James W. Mandell[8], Kevin S. Lee[1,2,9], Tajie H. Harris[1,2] & Jonathan Kipnis[1,2,3]



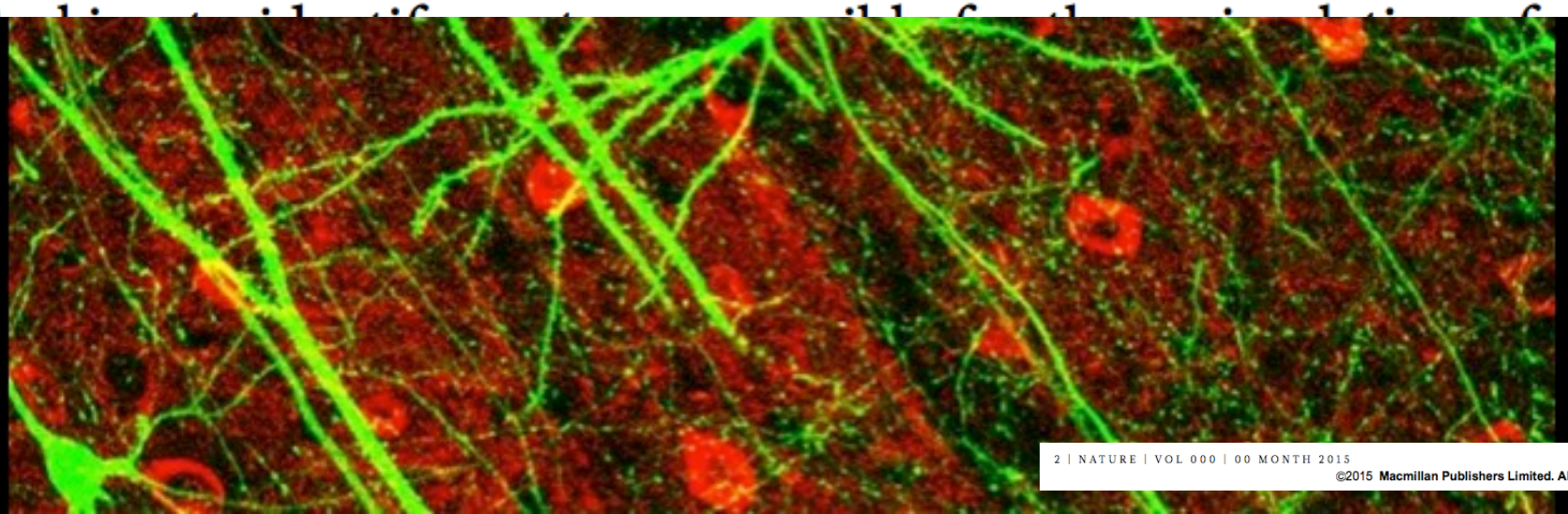


One of the characteristics of the central nervous system is the lack of a classical lymphatic drainage system. Although it is now accepted that the central nervous system undergoes constant immune surveillance that takes place within the meningeal compartment[1–3], the mechanisms governing the entrance and exit of immune cells from the central nervous system remain poorly understood[4–6]. In searching for T-cell gateways into and out of the



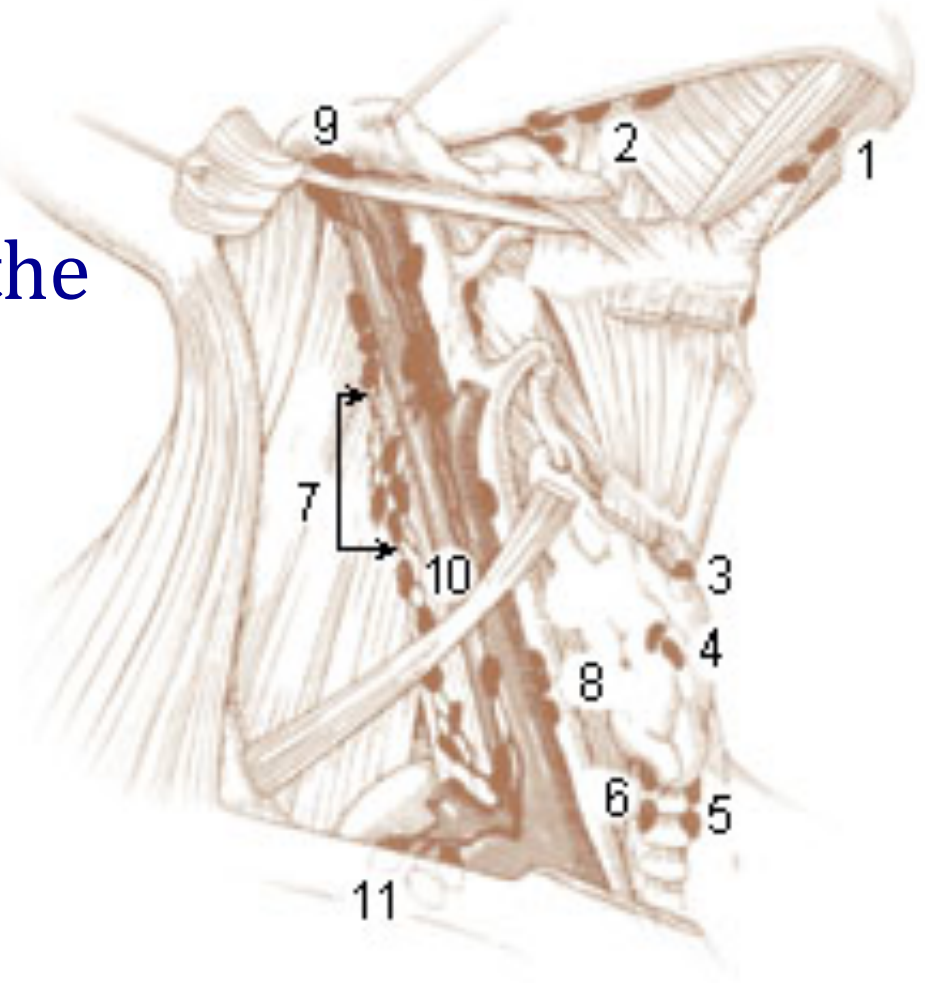


lature in the central nervous system. The discovery of the central nervous system lymphatic system may call for a reassessment of basic assumptions in neuroimmunology and sheds new light on the aetiology of neuroinflammatory and neurodegenerative diseases associated with immune system dysfunction.



The lymph coming from the brain drains in the deep cervical lymph nodes.

This is a group of cervical lymph nodes found near the internal jugular vein.



Intersecting the increased EAF volume observations in ASD with the EAF drainage to deep cervical lymph nodes draws our attention to the pathogenetic potential of chronic infections leading to inflammation and subsequent deficit in lymphatic drainage.

In other words,

- **Infection or inflammation** of the deep cervical nodes may lead to impaired lymph drainage with consequent accumulation of extra-axial fluid in the brain that leads to disruption of the connections between neurons and glial cells.

Supporting the role of chronic infection/inflammation in ASD pathogenesis, multiple polyomaviral infections were observed to be significantly more common in the post-mortem brains of ASD individuals (Lintas et al., 2010) and ASD individuals show immune transcriptome alterations in the temporal cortex that seem to indicate immune dysregulation with consequent inflammation (Garbett et al., 2008). Piras et al. (2014) correlated anti-brain antibodies with specific deficits in ASD, thus

Supporting the role of chronic infection/inflammation in ASD pathogenesis, multiple polyomaviral infections were observed to be significantly more common in the post-mortem brains of ASD individuals (Lintas et al., 2010) and ASD individuals show immune transcriptome alterations in the temporal cortex that seem to indicate immune dysregulation with consequent inflammation (Garbett et al., 2008). Piras et al. (2014) correlated anti-brain antibodies with specific deficits in ASD, thus

reinforcing the notion that chronic inflammation is a common denominator that may lead to EAF increase because of impaired meningeal lymphatic drainage.

World J Biol Psychiatry. 2002 Jul;3(3):162-6.

Treatment of late onset autism as a consequence of probable autommune processes related to chronic bacterial infection.

Matarazzo EB[1].

Author information

Abstract

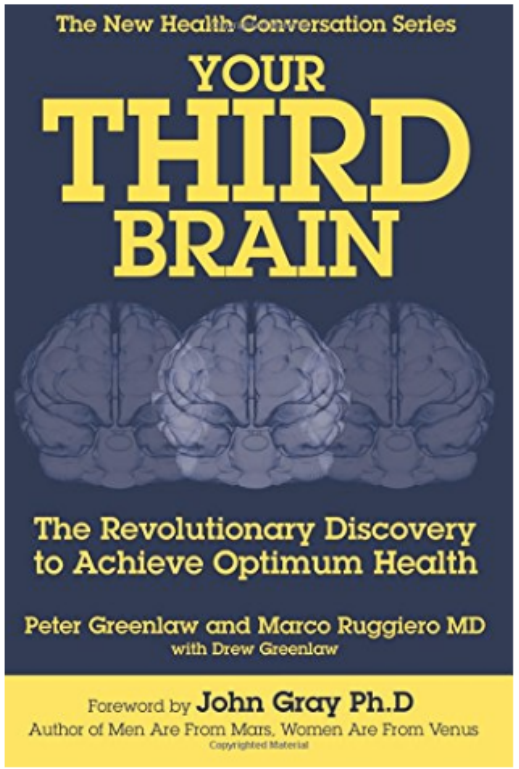
Two cases are described of children who at first developed normally, but before the age of three developed autistic symptoms following the reactivation of a chronic oto-rhinolaryngologic infection. The clinical and laboratory data of the cases support the aetiological hypothesis of an autoimmune process. Adrenocorticotrophic hormone (ACTH), prescribed in the first months of the disease, cured one case. The other patient, who was two years old when autistic symptoms appeared and was treated only six years later, showed a partial but definitive improvement with the immunosuppressive treatment. This report proposes that reactivation of a chronic bacterial infection be included among the aetiologies of Late Onset Autism, and demonstrates that, when the aetiological hypothesis of an autoimmune process based on clinical and laboratory data is considered, an immunosuppressive treatment, particularly with ACTH, can be very effective and also safe.

PMID: 12478882 [PubMed - indexed for MEDLINE]

- Impaired lymphatic drainage may result in the accumulation of metabolites (toxins) in the brain and in constant inflammation of the brain and the meninges with consequent alterations of brain development.

In conclusion, the observation by Louveau et al. (2015) leads us to hypothesize that meningeal lymphatic drainage deficit due to peripheral chronic infection/inflammation may be responsible for increased EAF and cortical dysplasia in ASD individuals and, possibly, for some of the symptoms typical of the disorder.

- Impaired lymphatic drainage in turn may decrease the immunological defence of the brain and its capability to fight pathogenic microbes that penetrate into the brain, mainly from the intestine.
- In other words, it may alter the **brain microbiome**.



As some of you may know,

- I have some experience in immunodeficiency, HIV and AIDS since I published a seminal paper on the AIDS epidemic together with Prof. Peter Duesberg of the University of California at Berkeley.

Review - Basic and Applied Anatomy and Embryology

AIDS since 1984: No evidence for a new, potentially lethal epidemic – not even in Africa

Peter H. Duesberg[1,*], Daniele Mandrioli[1], Amanda McCormack[1], Joshua M. Nicholson[2], David Rasnick[3], Christian Fiala[4], Claus Koehnlein[5], Henry H. Bauer[2] and Marco Ruggiero[6]

OPEN ACCESS Freely available online

PLOS ONE

Brain Microbial Populations in HIV/AIDS: α-Proteobacteria Predominate Independent of Host Immune Status

William G. Branton[1], Kristofor K. Ellestad[2], Ferdinand Maingat[1], B. Matt Wheatley[3], Erling Rud[4], René L. Warren[5], Robert A. Holt[5], Michael G. Surette[6], Christopher Power[1,2]*

1 Department of Medicine (Neurology), University of Alberta, Edmonton, Alberta, Canada, **2** Department of Medical Microbiology & Immunology, University of Alberta, Edmonton, Alberta, Canada, **3** Department of Surgery, University of Alberta, Edmonton, Alberta, Canada, **4** National Laboratory for HIV Pathogenesis, Health Canada, Ottawa, Ontario, Canada, **5** British Columbia Cancer Agency, Genome Sciences Centre, Vancouver, British Columbia, Canada, **6** Department of Medicine, McMaster University, Hamilton, Ontario, Canada

Introduction

The existence of commensal microbes that colonize organs within the human body has long been recognized and termed the microbiome [1,2]. Once thought of as harmless tenants, it has

In an organ widely assumed to be free of infectious agents in the absence of a specific disease process, autopsied and surgically-derived human brain specimens showed a restricted but distinct bacterial population in the present studies, which was composed of bacterial classes chiefly recognized in the physical environment, i.e., soil and water. The sources of these agents might include oral consumption or inhalation with eventual transport to the brain as intracellular agents in activated leukocytes trafficking into the brain. The brain is constantly surveyed by trafficking leukocytes (activated lymphocytes and macrophages), which provide a Trojan horse mechanism for microbial entry into the nervous system across the blood brain barrier. In fact, this mechanism is well

In other words,

- In the brain there are microbes that are commonly found in soil and water.
- The cells of the immune system (**macrophages**) carry these microbes to the brain.
- The brain lymphatic system, through which the immune cells travel, is instrumental in carrying the good or the bad microbes to the brain and **influence its function**.

patients, which are heavily infiltrated with blood derived leuko-
cytes [42]. Since bacteria express multiple molecules that activate immune signaling cascades by engaging Toll- or NOD-like receptors, etc., their capacity for influencing brain function [43] is immense. Hence, studies focused on delineating the brain's microbiome at the species level together with their individual effects on host cell physiology might lead to a greater understanding of human neurobiology including cognitive, motor, sensory and behavioral functions.

We now have to deal with a totally new concept:

The Brain Microbiome

and

Autism

The role of the **gut** microbiome

- In autism is very well acknowledged.

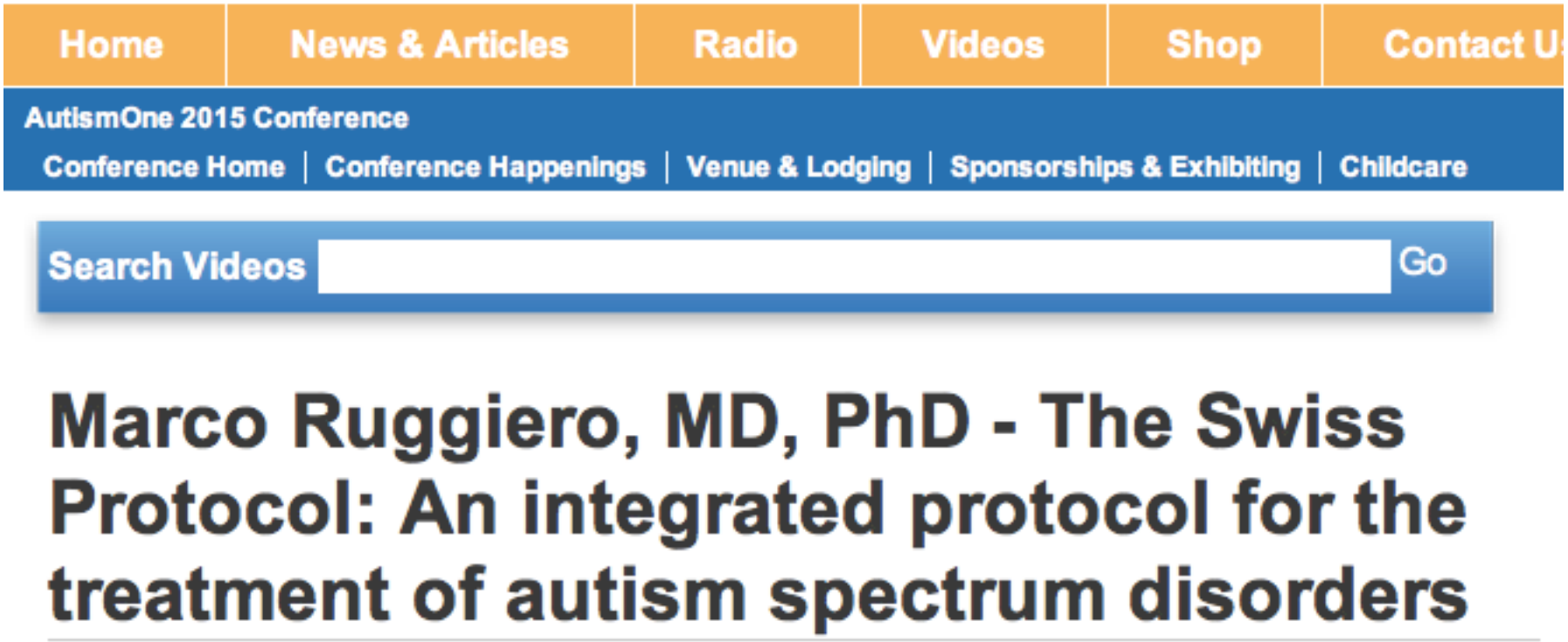
Published in final edited form as:
Curr Psychiatry Rep. 2013 February ; 15(2): 337. doi:10.1007/s11920-012-0337-0.

The Gut Microbiome: A New Frontier in Autism Research

Jennifer G. Mulle[1,4], **William G. Sharp**[2,3], and **Joseph F. Cubells**[4,5]
[1]Department of Epidemiology, Emory University Rollins School of Public Health
[2]Department of Pediatrics, Emory University School of Medicine
[3]Marcus Autism Center, Children's Healthcare of Atlanta
[4]Department of Human Genetics, Emory University School of Medicine
[5]Emory Autism Center, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine

The following two slides

- Were presented at AutismOne 2015.



We now have all the elements to develop an **integrative protocol** that targets the **brain**, the **immune system**, the **gut** and the **microbiome**.

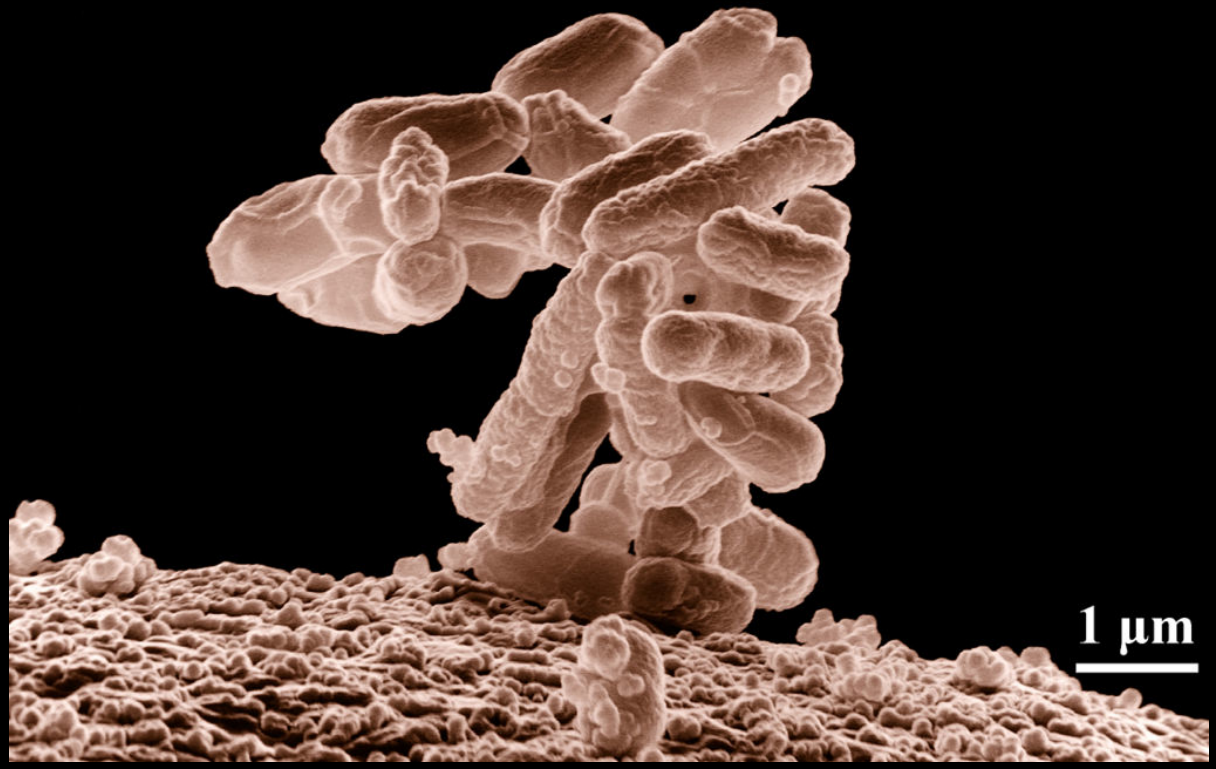
- All these organs and systems are compromised to some extent in autism, and strategies targeting each one of these organs proved somehow effective.
- However, the brain, the immune system, the gut and the microbiome **are not separate organs**, but different anatomical and functional components of the **same organ**.
- Therefore, we developed an integrative protocol that targets all these components **at the same time**.

In the past year,

- We have learned that the interconnection is even stricter than we had imagined.
- **Microbes are cells of the brain**, just like neurons and glial cells.
- Microbes are as important as neurons and glial cells.
- Microbes arrive to the brain through the immune system (**macrophages**).
- *The microbes that you have in the intestine are the microbes that you have in the brain*.

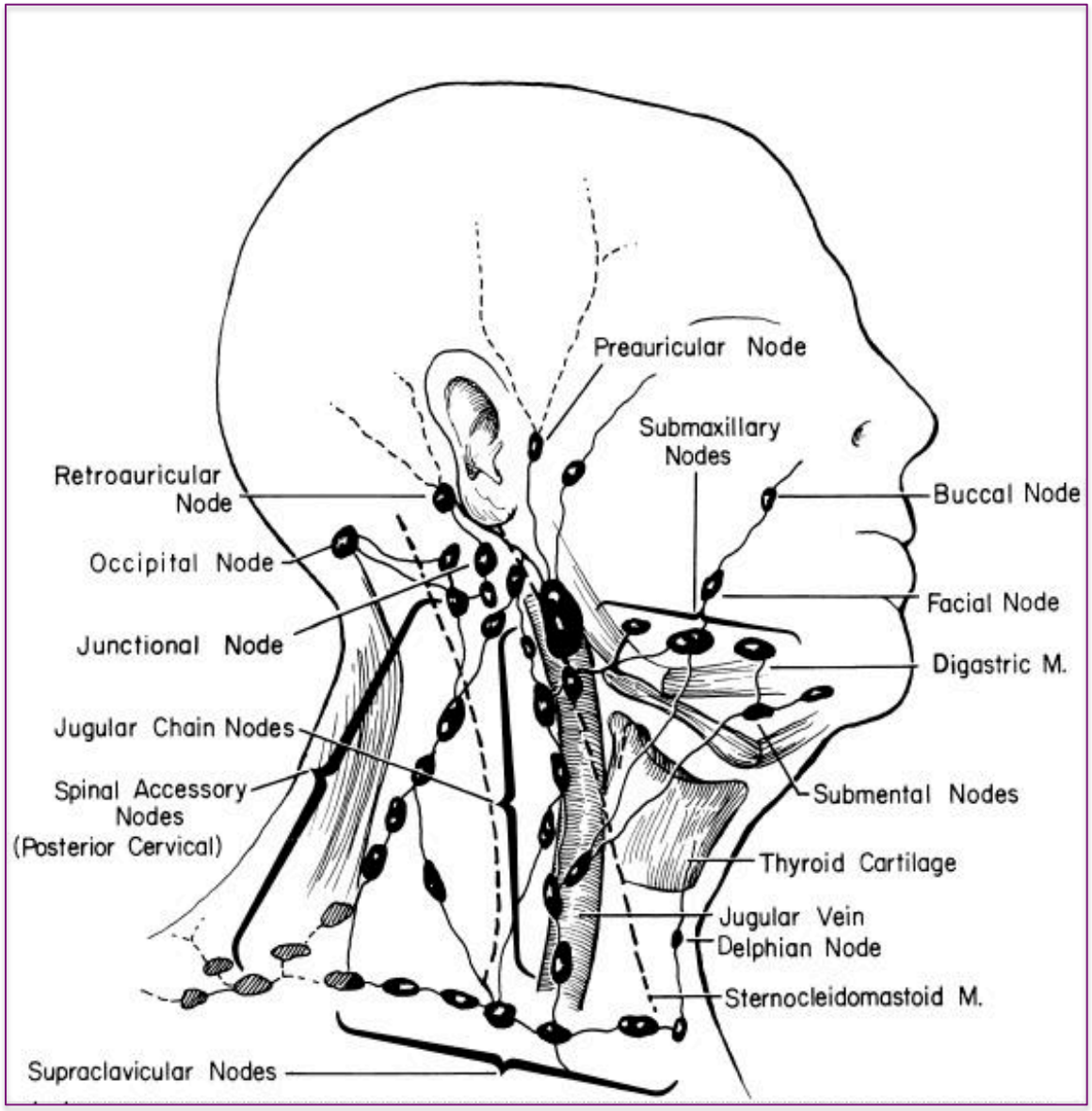
You want to have

- good microbes in your brain and, therefore, you want to have good microbes in your gut.



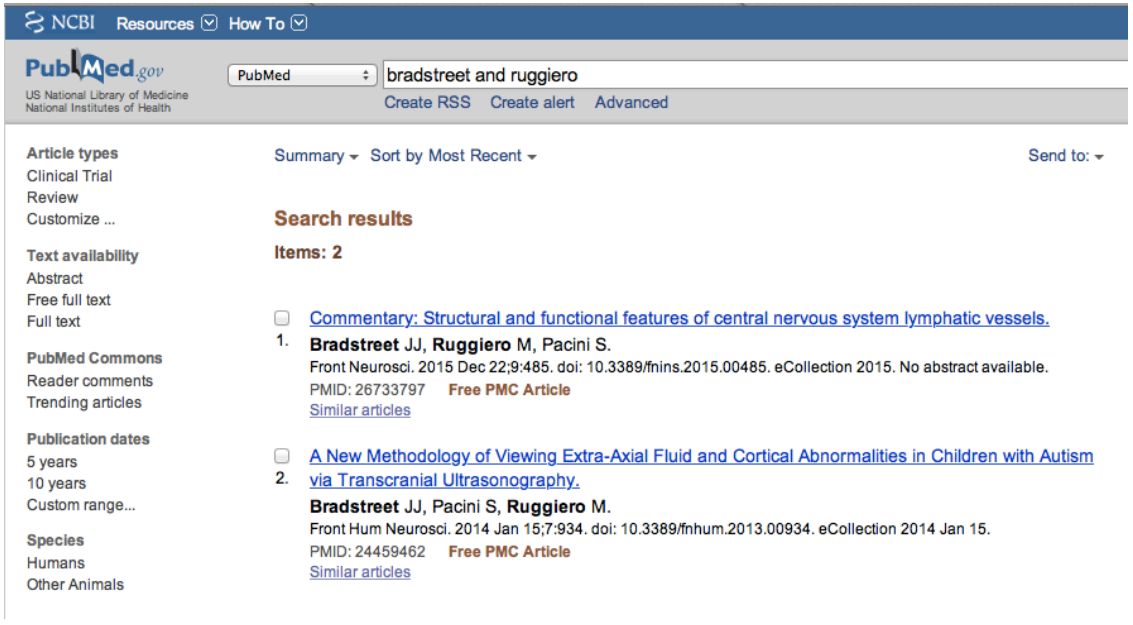
In addition,

- You want a functioning immune system inside your brain as well as a functional lymphatic drainage.



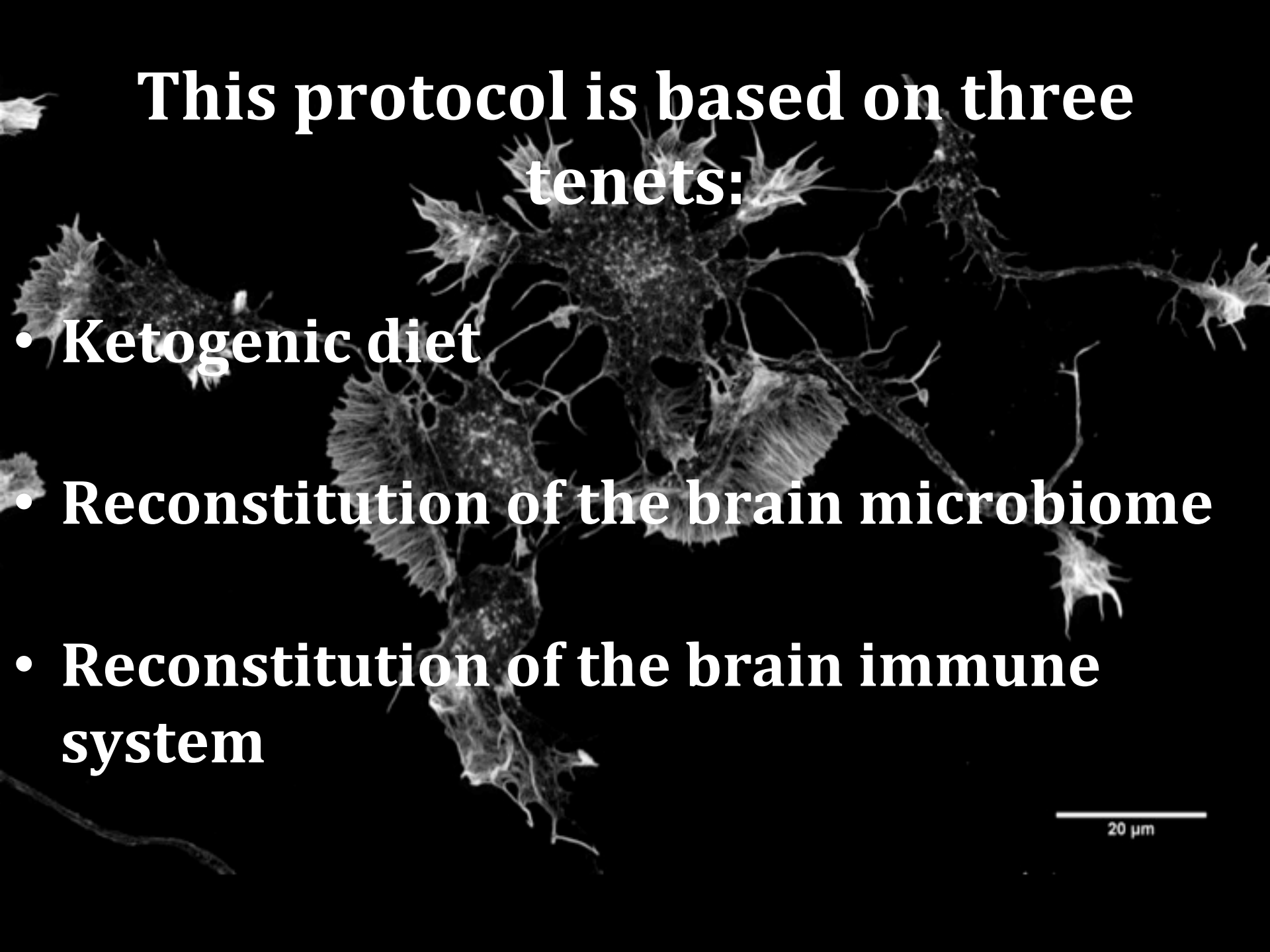
The natural, nutrition-based integrated protocol

- is based on the most recent scientific discoveries outlined in the previous slides including the recent papers by Bradstreet, Ruggiero and Pacini.



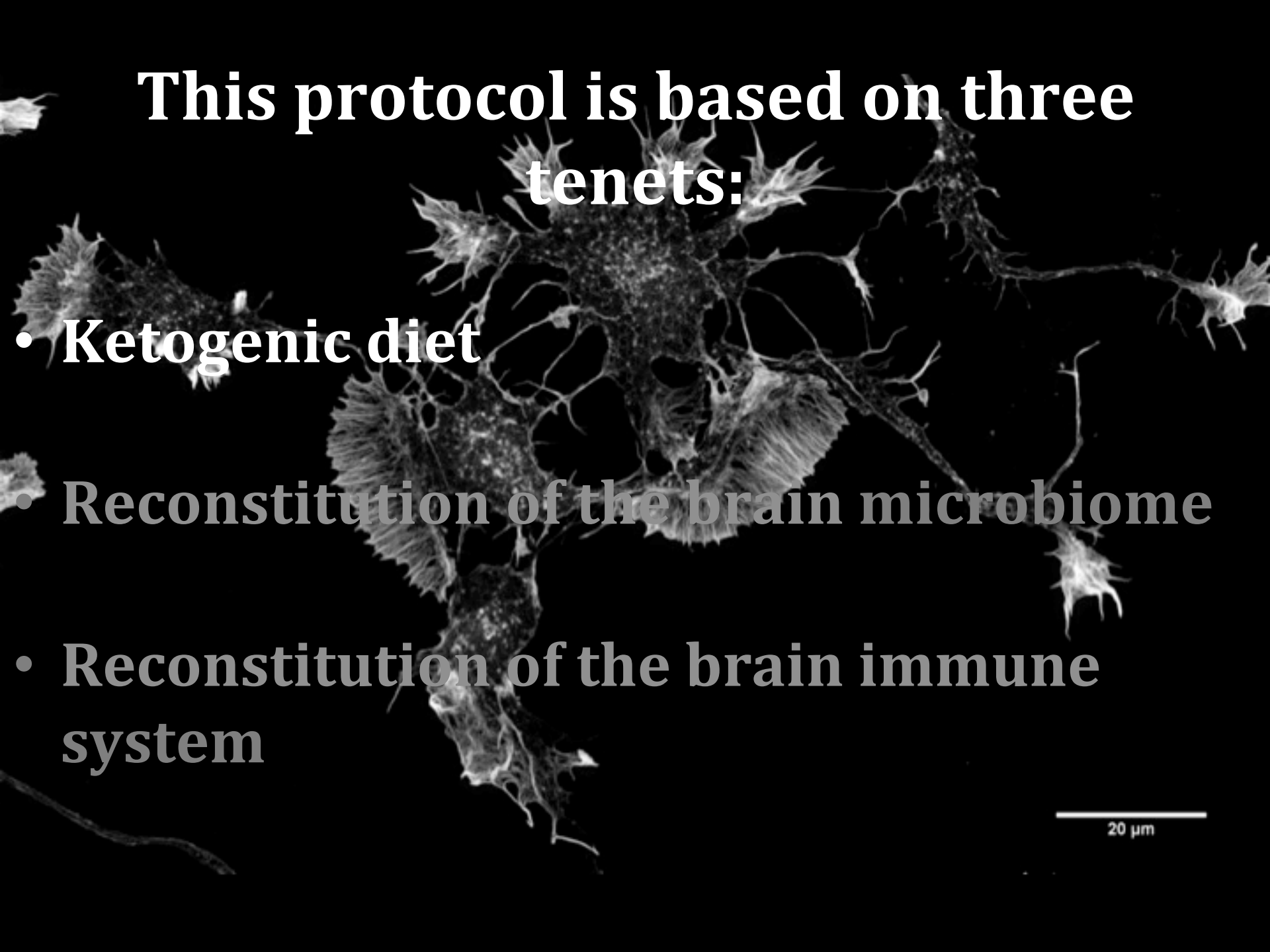
This protocol is based on three tenets:

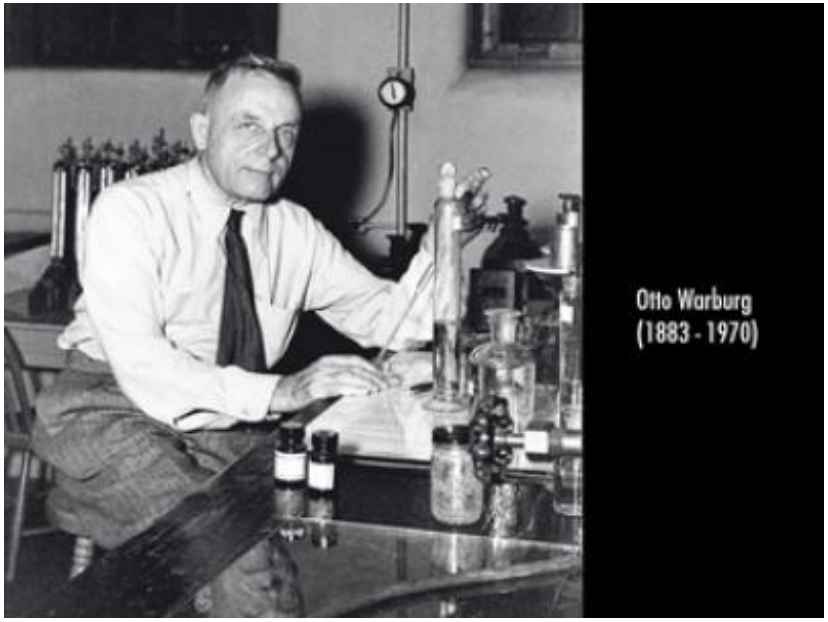
- Ketogenic diet
- Reconstitution of the brain microbiome
- Reconstitution of the brain immune system



This protocol is based on three tenets:

- **Ketogenic diet**
- **Reconstitution of the brain microbiome**
- **Reconstitution of the brain immune system**





ELSEVIER

journal homepage

Opinion paper

Toward a cancer-specific diet

Federico Bozzetti [a,*], Beth Zupec-Kania [b]

[a] *Faculty of Medicine, University of Milan, Italy*
[b] *The Charlie Foundation, Milwaukee, WI, USA*

ELSEVIER

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Review Article

Ketogenic diets as an adjuvant cancer therapy: History and potential mechanism

Bryan G. Allen *[,1], Sudershan K. Bhatia [1], Carryn M. Anderson, Julie M. Eichenberger-Gilmore, Zita A. Sibenaller, Kranti A. Mapuskar, Joshua D. Schoenfeld, John M. Buatti, Douglas R. Spitz, Melissa A. Fath

Free Radical and Radiation Biology Program, Department of Radiation Oncology, Holden Comprehensive Cancer Center, University of Iowa, Iowa City, IA 52242, USA

frontiers in
PEDIATRICS

PERSPECTIVE ARTICLE
published: 30 June 2014
doi: 10.3389/fped.2014.00069

Potential therapeutic use of the ketogenic diet in autism spectrum disorders

Eleonora Napoli [1] ****, Nadia Dueñas*** [1] ***and Cecilia Giulivi*** [1,2]

[1] *Department of Molecular Biosciences, University of California Davis, Davis, CA, USA*
[2] *Medical Investigations of Neurodevelopmental Disorders (M. I. N. D.) Institute, Sacramento, CA, USA*

Edited by:
Roberto Canitano, University Hospital of Siena, Italy

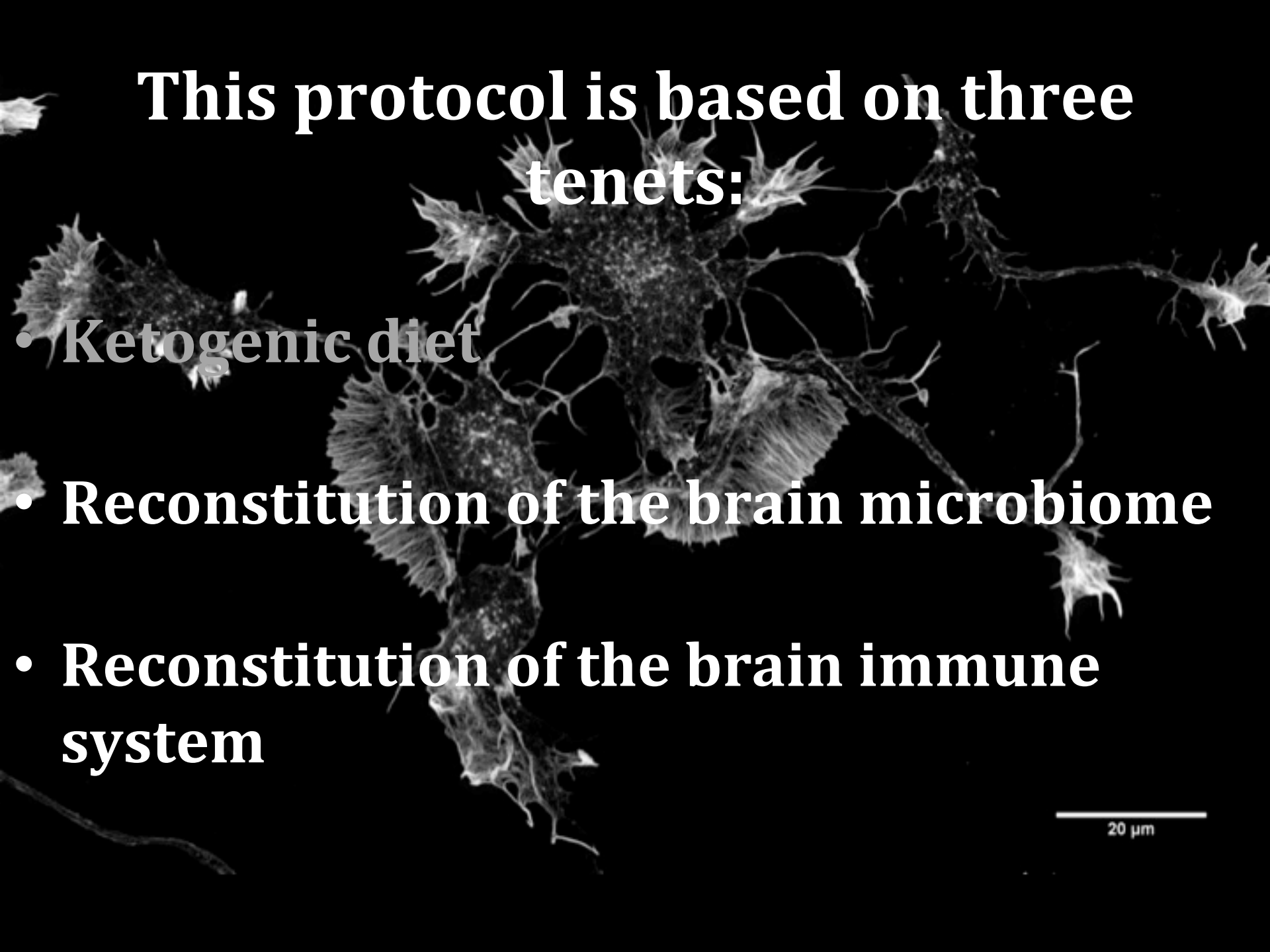
Reviewed by:
Richard Eugene Frye, Children's Hospital Boston/Harvard University, USA
Daniel Rossignol, Rossignol Medical Center, USA

****Correspondence:***
Eleonora Napoli, Department of Molecular Biosciences, University of California Davis, One Shields Avenue,

The ketogenic diet (KGD) has been recognized as an effective treatment for individuals with glucose transporter 1 (GLUT1) and pyruvate dehydrogenase (PDH) deficiencies as well as with epilepsy. More recently, its use has been advocated in a number of neurological disorders prompting a newfound interest in its possible therapeutic use in autism spectrum disorders (ASD). One study and one case report indicated that children with ASD treated with a KGD showed decreased seizure frequencies and exhibited behavioral improvements (i.e., improved learning abilities and social skills). The KGD could benefit individuals with ASD affected with epileptic episodes as well as those with either PDH or mild respiratory chain (RC) complex deficiencies. Given that the mechanism of action of the KGD is not fully understood, caution should be exercised in ASD cases lacking a careful biochemical and metabolic characterization to avoid deleterious side effects or refractory outcomes.

This protocol is based on three tenets:

- Ketogenic diet
- Reconstitution of the brain microbiome
- Reconstitution of the brain immune system



Until recently,

- Our main target, as far as reconstituting the microbiome was concerned, was the **gut** microbiome.
- We knew that by reconstituting the gut microbiome, we also rebalanced the immune system.
- Now, we know that we have to **reconstitute the brain microbiome and the brain immune system**.

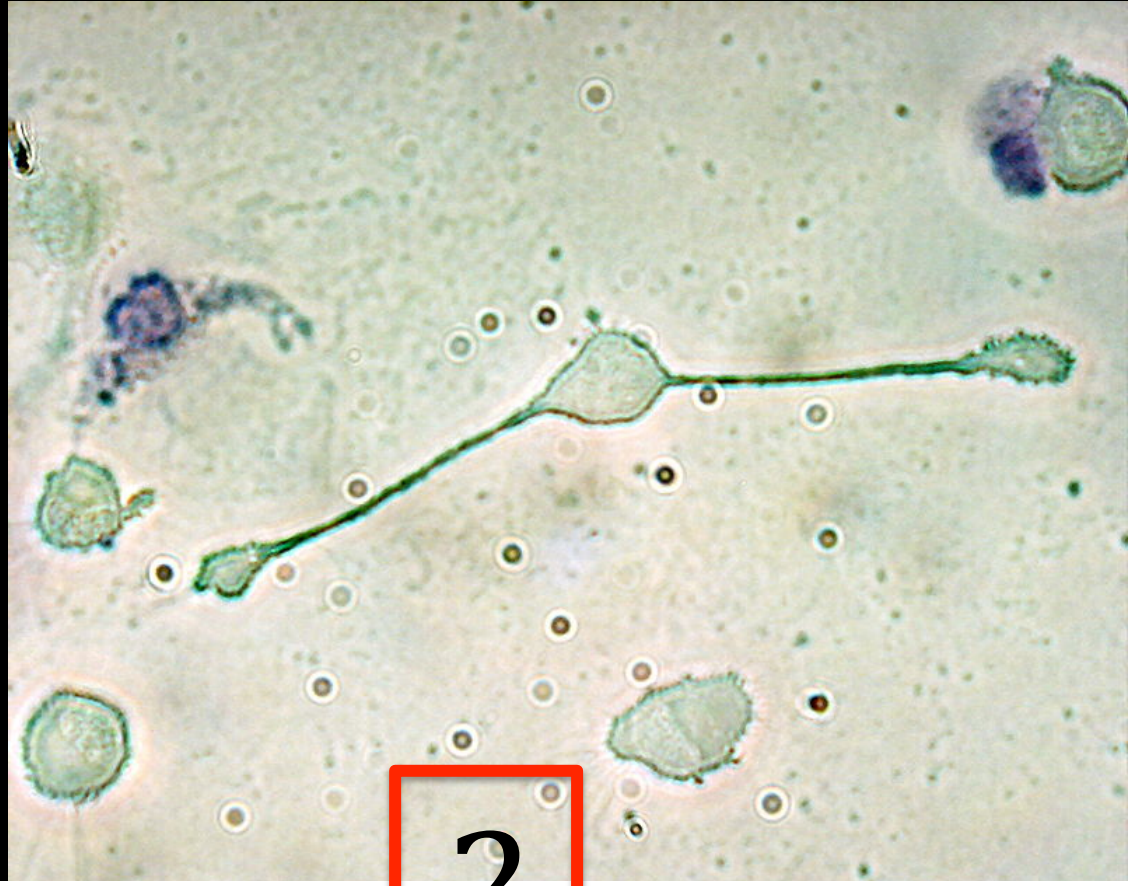
Having learned that

- microbes reach the brain using immune cells (**macrophages**) as vehicles, and knowing the pathways (lymphatic vessels) that the immune cells use to travel to the brain,
- we now have **all the elements** to reconstitute the brain microbiome as well as the brain immune system.

1

Élie Metchnikoff

By Agence Rol - Bibliothèque nationale de France, Public Domain.



2

Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5613–5617, August 1990
Cell Biology

Macrophage-colony-stimulating factor (CSF-1) induces proliferation, chemotaxis, and reversible monocytic differentiation in myeloid progenitor cells transfected with the human c-*fms*/CSF-1 receptor cDNA

JACALYN H. PIERCE*, EDDI DI MARCO*, GEORGE W. COX†, DANIELA LOMBARDI*, MARCO RUGGIERO*, LUIGI VARESIO†, LING MEI WANG‡, G. GHOSH CHOUDHURY‡, ALAN Y. SAKAGUCHI‡, PIER PAOLO DI FIORE*, AND STUART A. AARONSON*

*Laboratory of Cellular and Molecular Biology, National Cancer Institute, Building 37, Room 1E24, Bethesda, MD 20892; †Laboratory of Molecular Immunoregulation, National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701-1013; and ‡Department of Cellular and Structural Biology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284

Communicated by William E. Paul, April 13, 1990

CASSAT 5 22 15 1 James Bradstreet

AutismOne Media

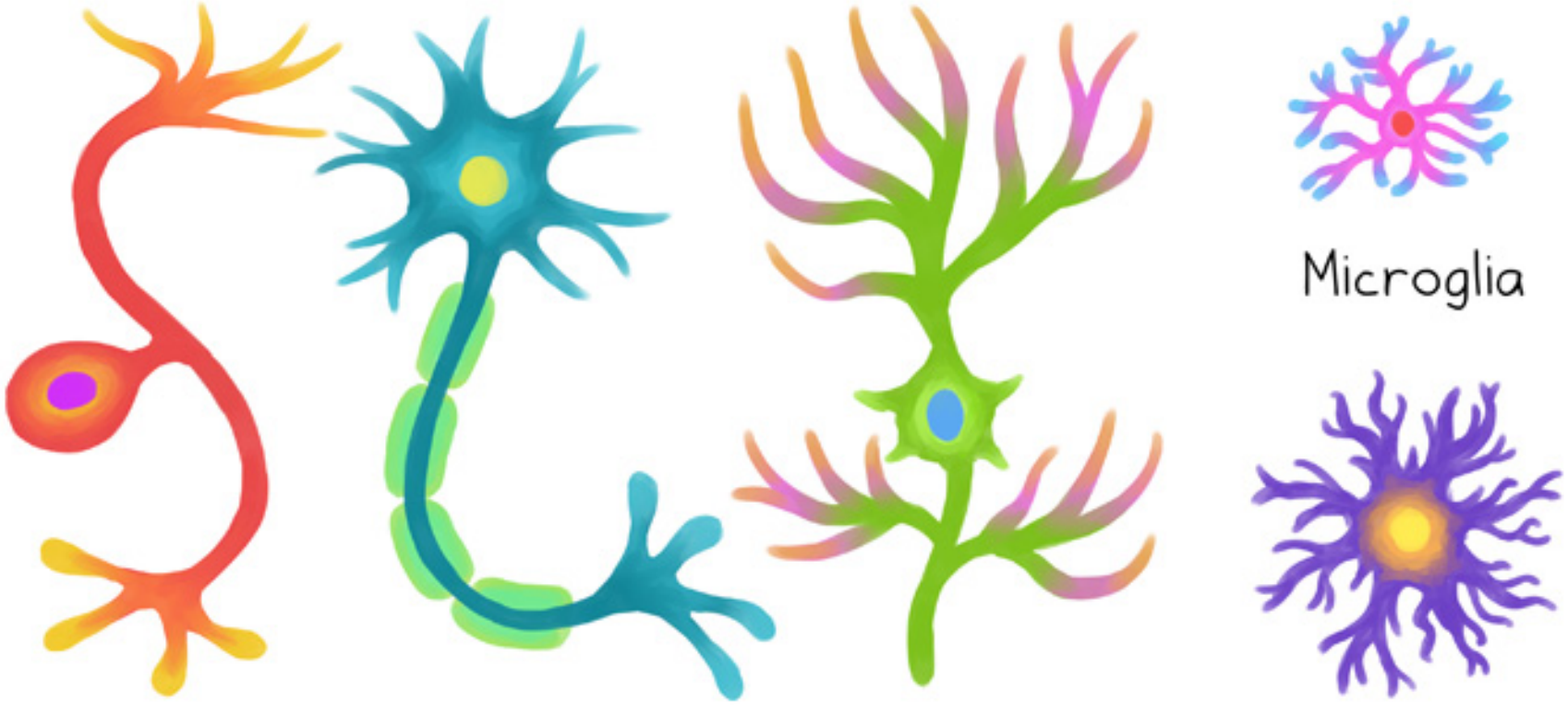
Iscriviti 1.719

12.129

Where is the experimental evidence

- Demonstrating that the actual reconstitution of the brain microbiome favours the re-establishment of neural networks?

Cells of the Central Nervous System



Sensory neuron

Motor neuron

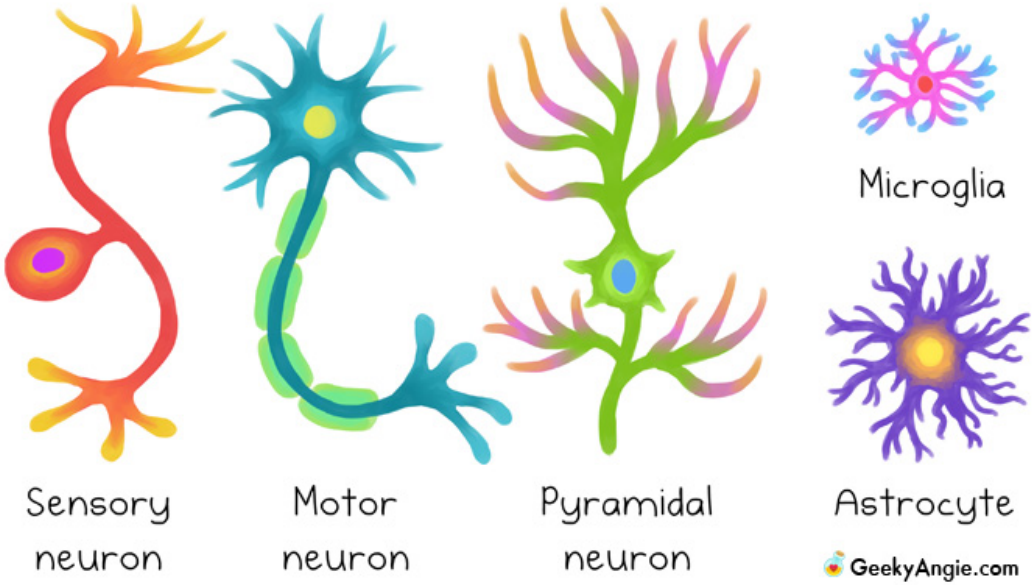
Pyramidal neuron

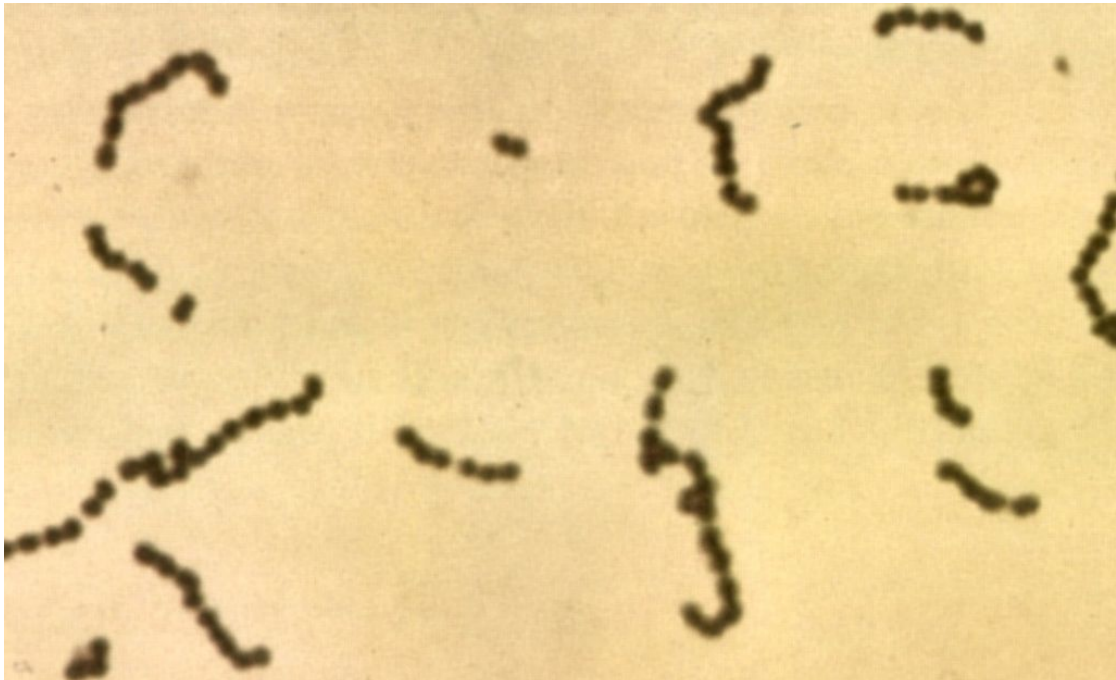
Microglia

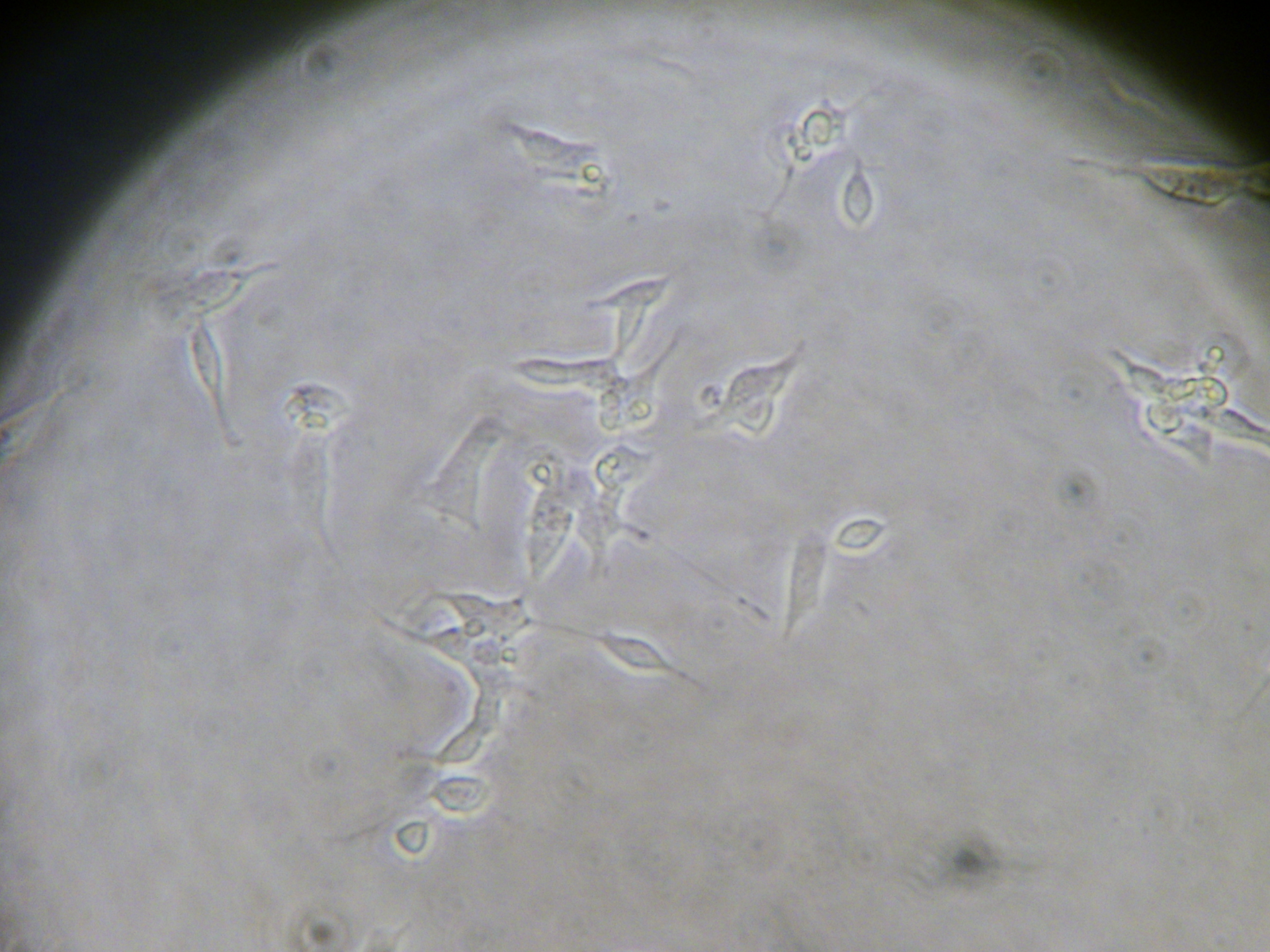
Astrocyte

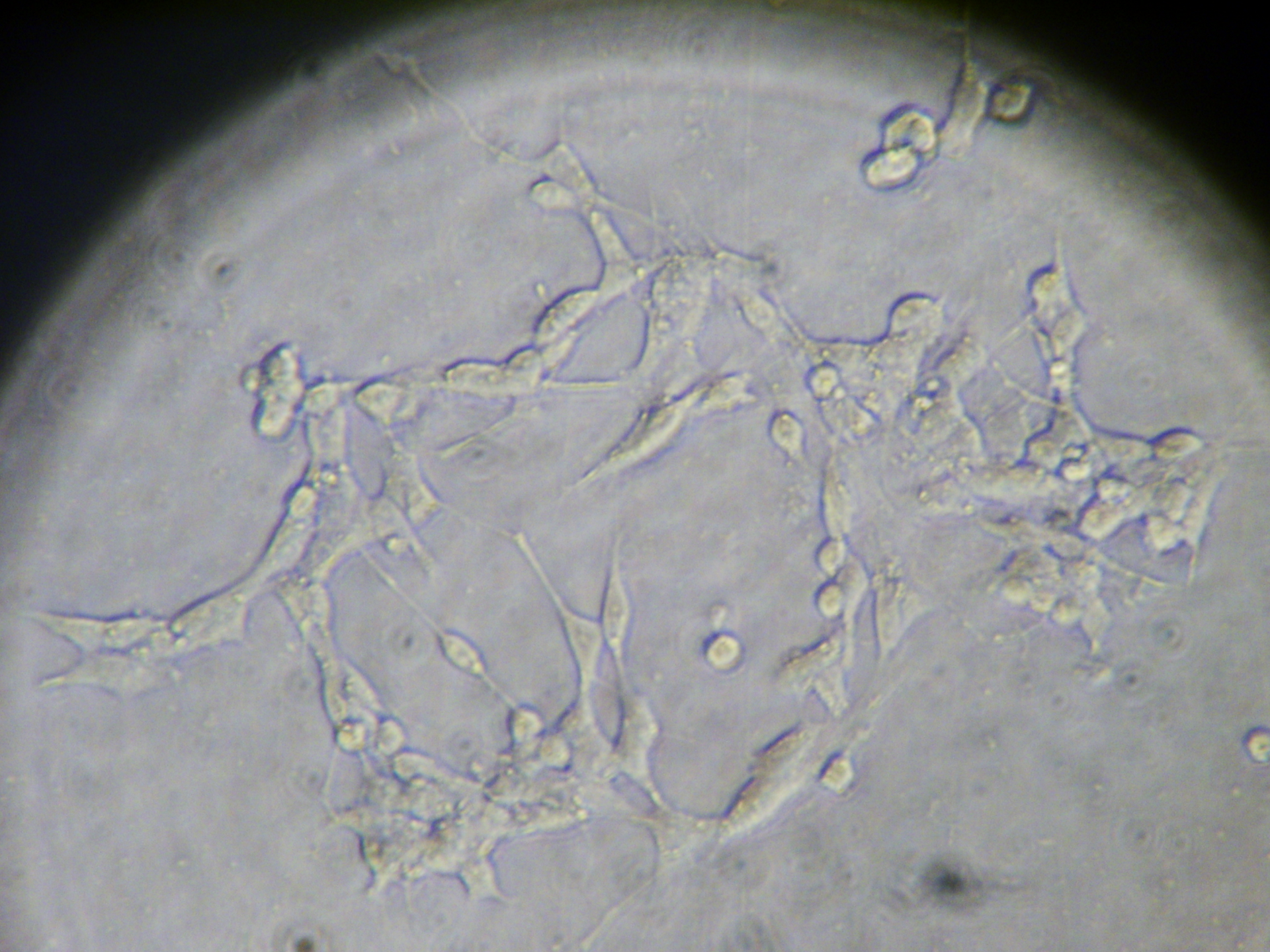
GeekyAngie.com

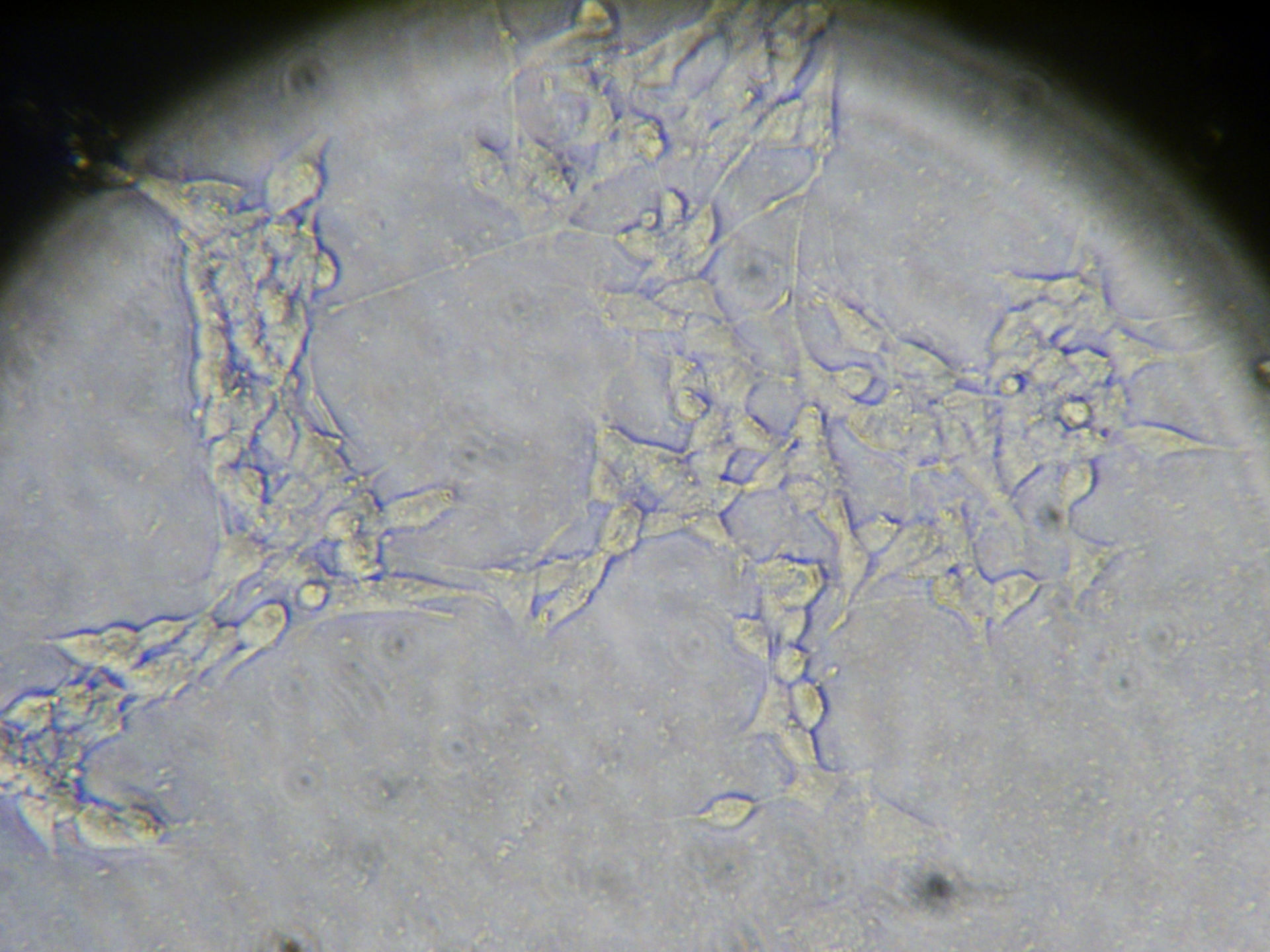
Cells of the Central Nervous System











These results are consistent with a recent study of ours where

- we have elucidated the molecular mechanism at the basis of the evolution of the human brain from primates to *homo sapiens sapiens*.
- We have observed that such an evolution is dependent on a gene, RUNX2, coding for a transcriptional factor that is regulated by nutrients and probiotics.

IJAE
Vol. 120, n. 1: 5-20, 2015
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Research Article - Embryology

Metopic suture and *RUNX2*, a key transcription factor in osseous morphogenesis with possible important implications for human brain evolution

Stefano Magherini[1], Maria Giulia Fiore[2], Brunetto Chiarelli[3], Antonio Serrao[2], Ferdinando Paternostro[1]*, Gabriele Morucci[1], Jacopo J.V. Branca[1], Marco Ruggiero[2], Stefania Pacini[1]

Departments of [1]Experimental and Clinical Medicine and of [2]Experimental Biomedical and Clinical Sciences and [3]Laboratory of Anthropology and Ethnology, Department of Evolutionary Biology, University of Firenze, Italy

Submitted October 25, 2013; accepted revised January 21, 2014

A

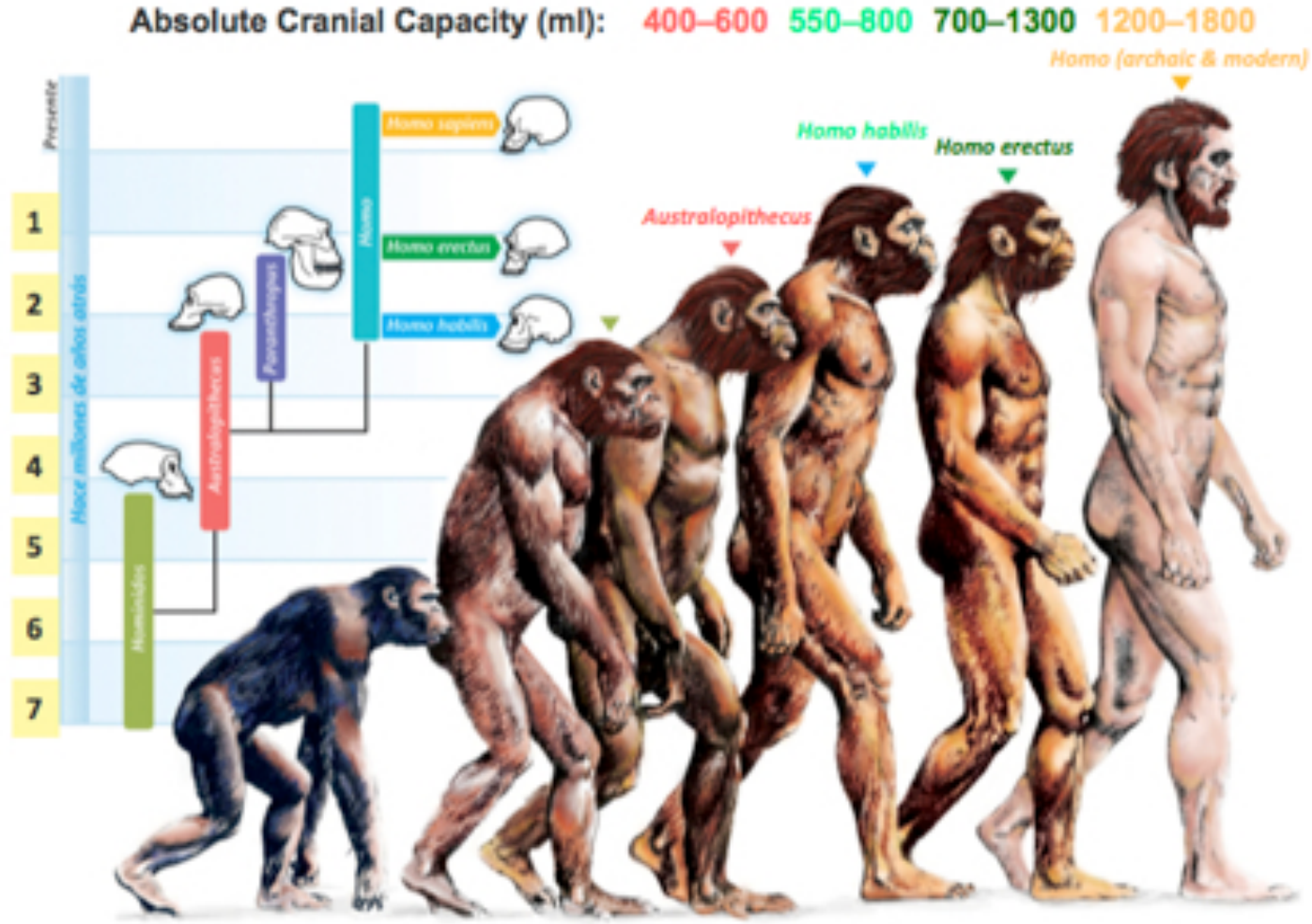
```
Homo sapiens isoform a      ------------------------------------------------------------
Homo sapiens isoform b      ------------------------------------------------------------
Pan troglodytes isoform 1   MLHSPHKQPQNHKCGANFLQEDSKKSLVFKWLISAGHYQPPRPTESFKAASSIYNRGYKF 60
Pan troglodytes isoform 4   MLHSPHKQPQNHKCGANFLQEDSKKSLVFKWLISAGHYQPPRPTESFKAASSIYNRGYKF 60
Gorilla gorilla isoform 1   MLHSPHKQPQNHKCGANFLQEDSKKSLVFKWLISAGHYQPPRPTESFKAASSIYNRGYKF 60
Gorilla gorilla isoform 2   MLHSPHKQPQNHKCGANFLQEDSKKSLVFKWLISAGHYQPPRPTESFKAASSIYNRGYKF 60
Macaca mulatta isoform 4    MLHSPHKQPQNHKCGANFLQEDSKKSLVFKWLISAGHYQPPRPTESFKAASSIYNRGYKF 60
Macaca mulatta isoform 6    MLHSPHKQPQNHKCGANFLQEDSKKSLVFKWLISAGHYQPPRPTESFKAASSIYNRGYKF 60

                                    exon I                  exon II
Homo sapiens isoform a      ---------MASNSLFSTVTPCQQNFFWDPSTSRRFSPPSSSLQPGKMSDVSPVVAAQQQQ 52
Homo sapiens isoform b      ---------MASNSLFSTVTPCQQNFFWDPSTSRRFSPPSSSLQPGKMSDVSPVVAAQQQQ 52
Pan troglodytes isoform 1   YLKKKGGTMASNSLFSTVTPCQQNFFWDPSTSRRFSPPSSSLQPGKMSDVSPVVAAQQQQ 120
Pan troglodytes isoform 4   YLKKKGGTMASNSLFSTVTPCQQNFFWDPSTSRRFSPPSSSLQPGKMSDVSPVVAAQQQQ 120
Gorilla gorilla isoform 1   YLKKKGGTMASNSLFSTVTPCQQNFFWDPSTSRRFSPPSSSLQPGKMSDVSPVVAAQQQQ 120
Gorilla gorilla isoform 2   YLKKKGGTMASNSLFSTVTPCQQNFFWDPSTSRRFSPPSSSLQPGKMSDVSPVVAAQQQQ 120
Macaca mulatta isoform 4    YLKKKGGTMASNSLFSTVTPCQQNFFWDPSTSRRFSPPSSSLQPGKMSDVSPVVAAQQQQ 120
Macaca mulatta isoform 6    YLKKKGGTM----------------------------------------------------
```

B

```
Homo sapiens isoform a      QQQQQQQQ
Homo sapiens isoform b      QQQQQQQQ
Pan troglodytes isoform 1   QQQQQQQQ
Pan troglodytes isoform 4   QQQQQQQQ
Gorilla gorilla isoform 1   QQQQQQQQ
Gorilla gorilla isoform 2   QQQQQQQQ
Macaca mulatta isoform 4    QQQQQQQQ
Macaca mulatta isoform 6    QQQQQQQQ
```

C

```
Homo sapiens isoform a      AQSSPPWS
Homo sapiens isoform b      AQSSPPWS
Pan troglodytes isoform 1   AQSSPPWS
Pan troglodytes isoform 4   AQSSPPWS
Gorilla gorilla isoform 1   AQSSPPWS
Gorilla gorilla isoform 2   AQSSPPWS
Macaca mulatta isoform 4    AQSSPPWS
Macaca mulatta isoform 6    AQSSPPWS
```



PLoS One. 2013 Dec 17;8(12):e83155. doi: 10.1371/journal.pone.0083155. eCollection 2013.

Probiotic supplementation promotes calcification in Danio rerio larvae: a molecular study.

Maradonna F[1], Gioacchini G[1], Falcinelli S[1], Bertotto D[2], Radaelli G[2], Olivotto I[1], Carnevali O[3].

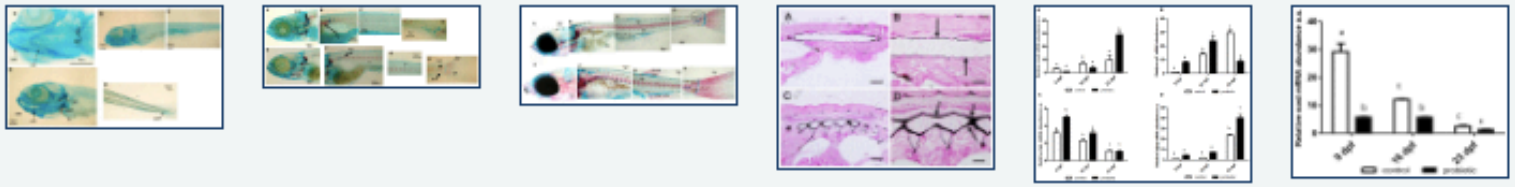
Author information

Abstract

A growing number of studies have been showing that dietary probiotics can exert beneficial health effects in both humans and animals. We previously demonstrated that dietary supplementation with Lactobacillus rhamnosus - a component of the human gut microflora - enhances reproduction, larval development, and the biomineralization process in Danio rerio (zebrafish). The aim of this study was to identify the pathways affected by L. rhamnosus during zebrafish larval development. Our morphological and histochemical findings show that L. rhamnosus accelerates bone deposition through stimulation of the expression of key genes involved in ossification, e.g. runt-related transcription factor 2 (runx2), Sp7 transcription factor (sp7), matrix Gla protein (mgp), and bone gamma-carboxyglutamate (gla) protein (bglap) as well as through inhibition of sclerostin (sost), a bone formation inhibitor. Western blot analysis of mitogen-activated protein kinase 1 and 3-(Mapk1 and Mapk3), which are involved in osteoblast and osteocyte differentiation, documented an increase in Mapk1 16 days post fertilization (dpf) and of Mapk3 23 dpf in individuals receiving L. rhamnosus supplementation. Interestingly, a reduction of sost detected in the same individuals suggests that the probiotic may help treat bone disorders.

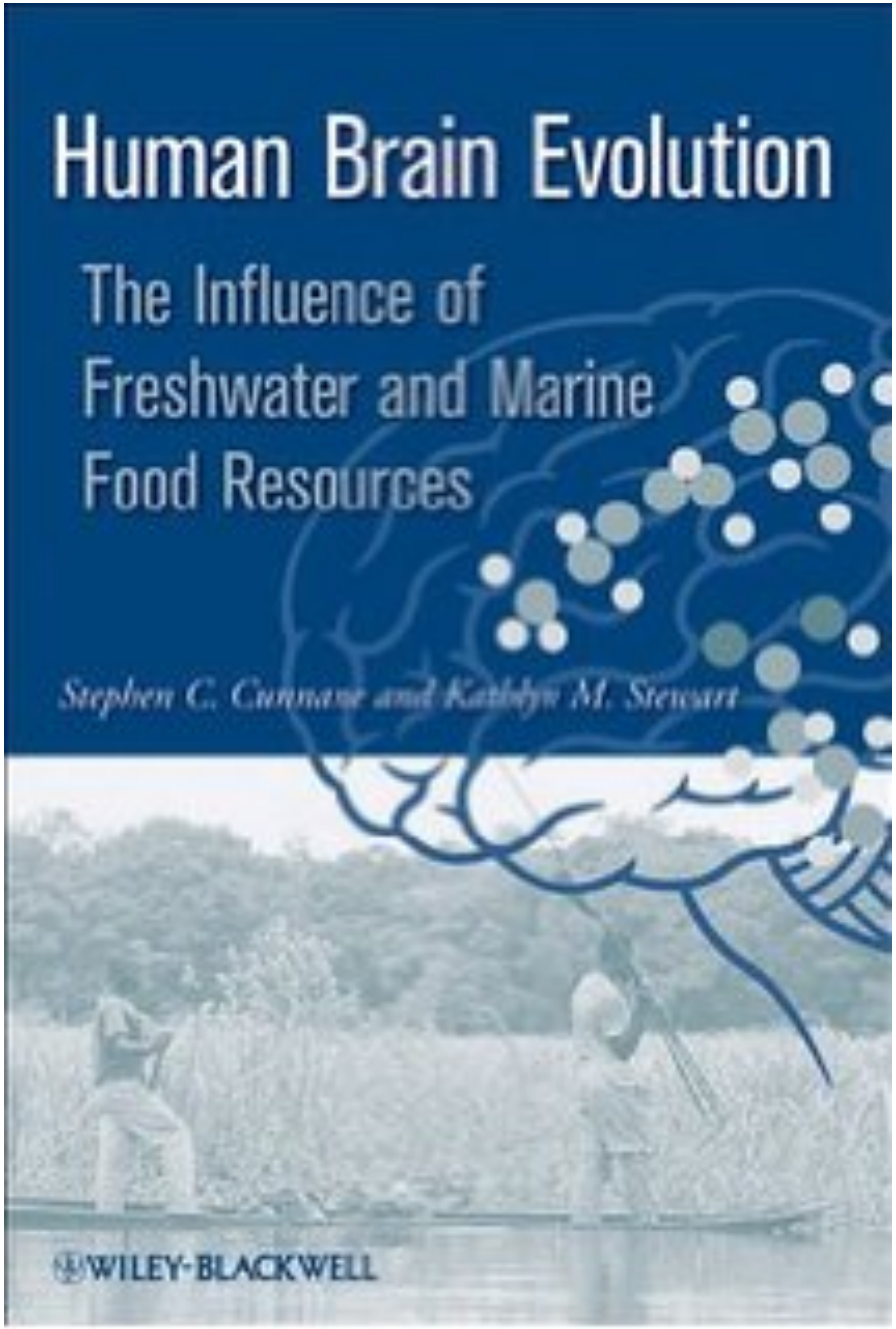
PMID: 24358259 [PubMed - indexed for MEDLINE] PMCID: PMC3866187 Free PMC Article

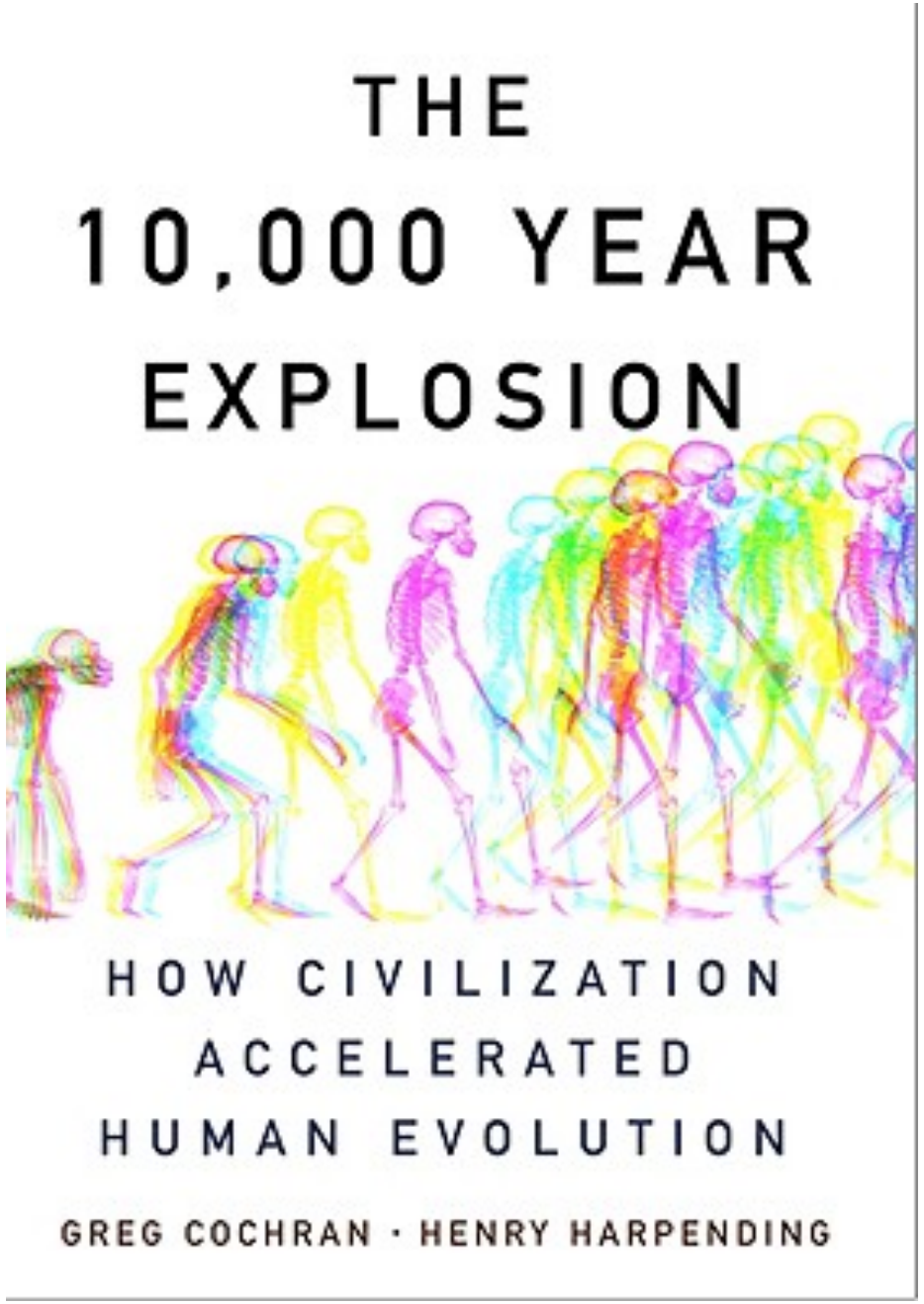
Images from this publication. See all images (8) Free text



In other words

- we demonstrated at the molecular level what had been hypothesized for years.
- The “explosive” recent evolution of the human brain is due to changes in nutrition and probiotics play a major role in this phenomenon.


Human Brain Evolution
The Influence of Freshwater and Marine Food Resources
Stephen C. Cunnane and Kathlyn M. Stewart
WILEY-BLACKWELL


THE
10,000 YEAR
EXPLOSION
HOW CIVILIZATION ACCELERATED HUMAN EVOLUTION
GREG COCHRAN · HENRY HARPENDING

Conclusions
and
Future developments

Acknowledgements

- Dr. Stefania Pacini.
- Dr. Jeff Bradstreet.
- Dr. Heinz Reinwald.
- Dr. Nicola Antonucci.
- Dr. Dario Siniscalco.
- Ms. Teri Arranga.
- Ms. Lorna Ortiz.
- All the families who gave us the strength and the courage to continue.