Base Hits

with Dr. Tom O'Bryan



Changing the Direction of Your Nervous System from Fight, Flight or Fright to Calm, Regenerative Healing

Session 3:

Fatigue

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It is not the stress itself, but how the body reacts or responds to that stress that is the problem.

Premise #1

In order to make it 'easy to do the right thing', we must understand how and why we've gotten to where we are, if we want to arrest and reverse dis-ease



Professor Alessio Fasano

- Professor of Pediatrics at Harvard Medical School
- Professor of Nutrition at Harvard T.H. Chan School of Public Health
- Chief of Pediatric Gastroenterology, Mass General Hospital
- Director, Mucosal Immunology Center, Harvard
- Director, Center for Celiac Research and Treatment
- Director, Mucosal Immunology and Biology Research Center;
- Associate Chief for Basic, Clinical and Translational Research
- 213 publications on pubmed.gov
- Identified zonulin as the protein activated in intestinal permeability





F1000Research 2020, 9(F1000 Faculty Rev):69 Last updated: 31 JAN 2020

REVIEW

All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases [version 1; peer review: 3 approved]

Alessio Fasano [1,2]

[1]Mucosal Immunology and Biology Research Center, Center for Celiac Research and Treatment and Division of Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital for Children, Boston, Massachusetts, USA
[2]European Biomedical Research Institute of Salerno, Salerno, Italy

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Abstract

Improved hygiene leading to reduced exposure to microorganisms has been implicated as one possible cause for the recent "epidemic" of chronic inflammatory diseases (CIDs) in industrialized countries. That is the essence of the hygiene hypothesis that argues that rising incidence of CIDs may be, at least in part, the result of lifestyle and environmental changes that have made us too "clean" for our own good, so causing changes in our microbiota. Apart from genetic makeup and exposure to environmental triggers, inappropriate increase in intestinal permeability (which may be influenced by the composition of the gut microbiota), a "hyper-belligerent" immune system responsible for the tolerance–immune response balance, and the composition of gut microbiome and its epigenetic influence on the host genomic expression have been identified as three additional elements in causing CIDs. During the past decade, a growing number of publications have focused on human genetics, the gut microbiome, and proteomics, suggesting that loss of mucosal barrier function, particularly in the gastrointestinal tract, may substantially affect antigen trafficking, ultimately influencing the close bidirectional interaction between gut microbiome and our immune system. This cross-talk is highly influential in shaping the host gut immune system function and ultimately shifting genetic predisposition to clinical outcome. This observation led to a re-visitation of the possible causes of CIDs epidemics, suggesting a key pathogenic role of gut permeability. Pre-clinical and clinical studies have shown that the zonulin family, a group of proteins modulating gut permeability, is implicated in a variety of CIDs, including autoimmune, infective, metabolic, and tumoral diseases. These data offer novel therapeutic targets for a variety of CIDs in which the zonulin pathway is implicated in their pathogenesis.

Keywords

Chronic inflammatory diseases, Gut permeability, microbiome, zonulin

Open Peer Review

Reviewer Status

	Invited Reviewers		
	1	2	3
version 1			

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1 **Xin M. Luo**, Virginia Tech, Blacksburg, USA
2 **Michael Mees**, Chulalongkorn University, Bangkok, Thailand
3 **Arul Jayaraman**, Texas A&M Health Science Center, Bryan, USA

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There is growing evidence that the elements of gut permeability, immune system response, and gut microbiome—together with genetic predisposition and exposure to environmental triggers— make the *"perfect storm"* for Chronic Inflammatory Disease development.

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the *"perfect storm"*
for Chronic Inflammatory Disease development.



the *"perfect storm"* for Chronic Inflammatory Disease development.

- Genetic Vulnerability
- Environmental triggers
- Altered Microbiome (Dysbiosis)
- Intestinal Permeability
- Systemic Immune Response (Innate and Adaptive)

If there is 1 concept, that becomes the foundational paradigm you look through, for all chronic inflammatory diseases, this is it





the *"perfect storm"* for Chronic Inflammatory Disease development.

- Genetic Vulnerability
- Environmental triggers
- Altered Microbiome (Dysbiosis)
- Intestinal Permeability
- Systemic Immune Response (Innate and Adaptive)

Although there are likely dozens of environmental triggers that have contributed to your specific inflammatory state, what's the 'Big Kahuna'?



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Reviewer Status ✓ ✓ ✓

Among the several potential intestinal luminal stimuli that can stimulate zonulin release (thus Intestinal Permeability), small exposure to large amounts of bacteria (and its exhaust LPS) and gluten, have been identified as the two most powerful triggers

gastrointestinal tract, may substantially affect antigen trafficking, ultimately influencing the close bidirectional interaction between gut microbiome and our immune system. This cross-talk is highly influential in shaping the host gut immune system function and ultimately shifting genetic predisposition to clinical outcome. This observation led to a re-visitation of the possible causes of CIDs epidemics, suggesting a key pathogenic role of gut permeability. Pre-clinical and clinical studies have shown that the zonulin family, a group of proteins modulating gut permeability, is implicated in a variety of CIDs, including autoimmune, infective, metabolic, and tumoral diseases. These data offer novel therapeutic targets for a variety of CIDs in which the zonulin pathway is implicated in their pathogenesis.

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Clinical Review & Education

JAMA | Review

Celiac Disease and Nonceliac Gluten Sensitivity
A Review

Maureen M. Leonard, MD, MMSc; Anna Sapone, MD, PhD; Carlo Catassi, MD, MPH; Alessio Fasano, MD

IMPORTANCE The prevalence of gluten-related disorders is rising, and increasing numbers of individuals are empirically trying a gluten-free diet for a variety of signs and symptoms. This review aims to present current evidence regarding screening, diagnosis, and treatment for celiac disease and nonceliac gluten sensitivity.

OBSERVATIONS Celiac disease is a gluten-induced immune-mediated enteropathy characterized by a specific genetic genotype (*HLA-DQ2* and *HLA-DQ8* genes) and autoantibodies (antitissue transglutaminase and antiendomysial). Although the inflammatory process specifically targets the intestinal mucosa, patients may present with gastrointestinal signs or symptoms, extraintestinal signs or symptoms, or both, suggesting that celiac disease is a systemic disease. Nonceliac gluten sensitivity is diagnosed in individuals who do not have celiac disease or wheat allergy but who have intestinal symptoms, extraintestinal symptoms, or both, related to ingestion of gluten-containing grains, with symptomatic improvement on their withdrawal. The

and the development of possible comorbidities.

JAMA. 2017;318(7):647-656. doi:10.1001/jama.2017.9730

CME Quiz at jamanetwork.com/learning

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Section Editors: Edward Livingston, MD, Deputy Editor, and Mary McGrae McDermott, MD, Senior Editor.

Previous studies have shown that gliadin (in wheat) can cause an immediate and transient increase in gut permeability. This process takes place <u>in all individuals</u> who ingest gluten.

Celiac disease is a chronic, small-intestinal immune-mediated enteropathy initiated by exposure to dietary gluten in genetically predisposed individuals and characterized by specific autoantibodies against tissue transglutaminase 2 (anti-tTG2), endomysium, and/or deamidated gliadin peptide.[1] Although up to 40% of the population carries the genotype *HLA-DQ2* or *HLA-DQ8*, which is required for the development of celiac disease, only 2% to 3% of *HLA-DQ2* or *HLA-DQ8* carriers subsequently develop celiac disease.[2] Celiac disease, once considered a relatively rare gastrointestinal condition affecting almost exclusively young white children, can develop at any age and can affect almost any race. Celiac disease was first described by Samuel Gee in 1887. Wheat was hypothesized as the possible offending agent by William Dicke in 1941.[3]

The epidemiology, clinical presentation, pathophysiology, and management of the disease have changed since its initial description. There is strong evidence that celiac disease is an autoimmune disease triggered by the ingestion of gluten present in wheat, barley, and rye in genetically predisposed individuals. The prevalence of celiac disease in the general population is 1%, with regional differences (**Table 1**).[4] Celiac disease can affect any human organ or tissue (Table 1 and **Table 2**).[6]

Nonceliac gluten sensitivity is a term used to describe individuals who have intestinal signs or symptoms, extraintestinal signs or symptoms, or both, related to ingestion of gluten-containing grains (Table 2), with improvement when these are removed from a patient's diet. The frequency of nonceliac gluten sensitivity is unknown owing to the lack of validated biomarkers, but it is thought to be more common than celiac disease. Wheat allergy, the third gluten-related disorder, which will not be addressed in this review, is defined as an adverse type-2 helper T-cell immunologic reaction to wheat proteins and typically presents soon after wheat

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ORIGINAL PAPERS

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Iva Brynychova[1, B–D, F], Iva Hoffmanova[2, E, F], Milos Dvorak[3, E, F], Pavlina Dankova[1, A, C, E, F]

Increased Expression of TLR4 and TLR7 but Not Prolactin mRNA by Peripheral Blood Monocytes in Active Celiac Disease*

[1] Department of Anthropology and Human Genetics, Faculty of Science, Charles University in Prague, Czech Republic
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[3] First Medical Faculty, Charles University in Prague and General University Hospital in Prague, Czech Republic

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation;

Gliadin fragments have been identified to activate the innate immune system *via* TLR4

clinical signs and symptoms of CD improve after the introduction of a gluten-free diet (GFD). The focus was on changes in mRNA expression of selected toll-like receptors (TLR2, TLR4, TLR7), stress cytokine prolactin (PRL), and pro- and anti-inflammatory cytokines (TNF-α, IL-6, IL-12, IL-10) in PBMs.

Material and Methods. The study involved 20 CD patients diagnosed according to the European Society for Pediatric Gastroenterology, Hepatology and Nutrition criteria and Marsh criteria: 10 recently-diagnosed cases (rCD) and 10 on a GFD for a minimum of one year. The control group comprised 10 age- and sex-matched healthy volunteers. PBMs from peripheral blood specimens were separated using immunomagnetic CD14+ beads. Total RNA was isolated using a standard commercial kit. Cytokine and TLR mRNA levels were quantified by relative qPCR with *PGK1* as a reference gene.

Results. Significantly higher expression of TLR4 and TLR7 mRNA was observed in PBMs from rCD patients compared to the healthy controls (1.63 times higher; $p < 0.05$). TLR7 mRNA levels in rCDs were also significantly elevated in comparison to the CD-GFD patients (2.11 times higher; $p < 0.01$). TNF-α mRNA expression tended to be higher in both groups of patients; by contrast, in IL-6 mRNA, a trend to a fourfold decrease was detected in PBMs from the CD-GFD subjects. IL-10, IL-12 and PRL levels did not differ among the groups.

Conclusions. The data suggest that the inflammatory process in rCD intestinal mucosa and submucosa reflecting enterocyte damage can be detected in PBMs in peripheral blood. Further, the cytokine and TLR expression profile in PBMs alters after one year of GFD treatment (**Adv Clin Exp Med 2016, 25, 5, 887–893**).

Key words: celiac disease, monocytes, cytokines, prolactin, innate immunity.

Celiac disease (CD) is defined as the loss of oral tolerance to gluten in genetically predisposed individuals; it is an organ-specific autoimmune disease with a prevalence of about 1% in Europe [1]. Clinical manifestation of the gluten-induced inflammatory response in celiac mucosa is highly variable and can occur both in childhood and in adulthood. The only possible treatment of CD is a lifelong gluten-free diet (GFD).

Imbalance in the innate immune mechanisms is known to contribute to the pathogenesis of CD.

* This study was funded by the Grant Agency of Charles University in Prague (grant 316211).

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Reviewer Status ✓ ✓ ✓

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version 1	✓	✓	✓

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Gluten is misinterpreted by the zonulin pathway as a potential harmful component of a microorganism.

https://f1000research.com/articles/9-69/v1

So it makes complete sense that BEFORE you begin spending thousands of dollars on well-thought out lines of exploration for environmental triggers, check 1st for the Big Kahunas (Gluten and LPS)





APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2004, p. 4187–4192 Vol. 70, No. 7
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Molecular Analysis of Shower Curtain Biofilm Microbes

Scott T. Kelley,[1]† Ulrike Theisen,[2]† Largus T. Angenent,[3]
Allison St. Amand,[2] and Norman R. Pace[2]*

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Households provide environments that encourage the formation of microbial communities, often as biofilms. Such biofilms constitute potential reservoirs for pathogens, particularly for immune-compromised individuals. One household environment that potentially accumulates microbial biofilms is that provided by vinyl shower curtains. Over time, vinyl shower curtains accumulate films, commonly referred to as "soap scum," which microscopy reveals are constituted of lush microbial biofilms. To determine the kinds of microbes that constitute shower curtain biofilms and thereby to identify potential opportunistic pathogens, we conducted an analysis of rRNA genes obtained by PCR from four vinyl shower curtains from different households. Each of the shower curtain communities was highly complex. No sequence was identical to one in the databases, and no identical sequences were encountered in the different communities. However, the sequences generally represented similar phylogenetic kinds of organisms. Particularly abundant sequences represented members of the α-group of proteobacteria, mainly *Sphingomonas* spp. and *Methylobacterium* spp. Both of these genera are

Several studies have shown that domestic water supplies can be a source of opportunistic infectious agents, and household plumbing accumulates numerous microorganisms

cate (20, 25, 37, 43). Potential of adventitious pathogens in households pose a particular threat to such patients (24). Households provide many environments in which microorganisms can thrive, often with the formation of biofilms. Bacteria have been cultured from many environments in and around homes, particularly in moist settings such as those involving water pipes, toothbrushes, and spas (12, 14, 29, 30, 38). Although evidence of microbial growth and biofilm formation is ubiquitous in households, little is known about the diversity and complexity of the organisms that make up household microbial communities.

Several studies have shown that domestic water supplies can be a source of opportunistic infectious agents, and household plumbing accumulates numerous microorganisms (5, 55). Potentially infectious agents such as *Mycobacterium* spp. and *Legionella* spp. have been detected in water systems and may serve as reservoirs for infection (10, 24, 32, 33). One water-related setting that may provide a persistent reservoir for

several such communities. In our study, we isolated microbial-community DNA from shower curtains and used a PCR-based molecular survey to determine the phylogenetic diversity of 16S rRNA gene sequences from these communities. Numerous studies have utilized 16S rRNA sequences to assess the nature of microbial organisms in the environment without the requirement for culture (1, 2, 22, 39). The rRNA sequence collection constitutes a rough census of this particular community. The phylogenetic identification of the constituent microbes can provide some insight into their natures by comparison with available cultured organisms.

MATERIALS AND METHODS

Samples. DNA was extracted from five different samples taken from four different vinyl shower curtains, all in use for more than 6 months in Boulder, Colo. These samples consisted of (i) two samples from the same shower curtain, one from the bottom section (whitish pink flakes) of a dry shower curtain that had been stored at 25°C for about 1 week subsequent to wetting (SC1A) and one sample from a pink film on a corner of the curtain that was folded over and constantly wet (SC1B); (ii) one sample of white flakes from a dry curtain that had been stored at 25°C for about 1 week subsequent to a previous wetting (SC2); (iii) one sample of pinkish flakes from the bottom section of a dry curtain that had been stored at 25°C for about 1 week subsequent to wetting (SC3); and (iv) one sample of a pinkish orange biofilm from a wet shower curtain that was often used and essentially continuously moist (SC4). Dry biofilm flakes or moist bio-

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† S.T.K. and U.T. contributed equally to this work.

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Molecular Analysis of Shower Curtain Biofilm Microbes

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Shower curtains are a potential source of opportunistic pathogens associated with biofilms. Frequent cleaning or disposal of shower curtains is indicated, particularly in households with immune-compromised individuals.

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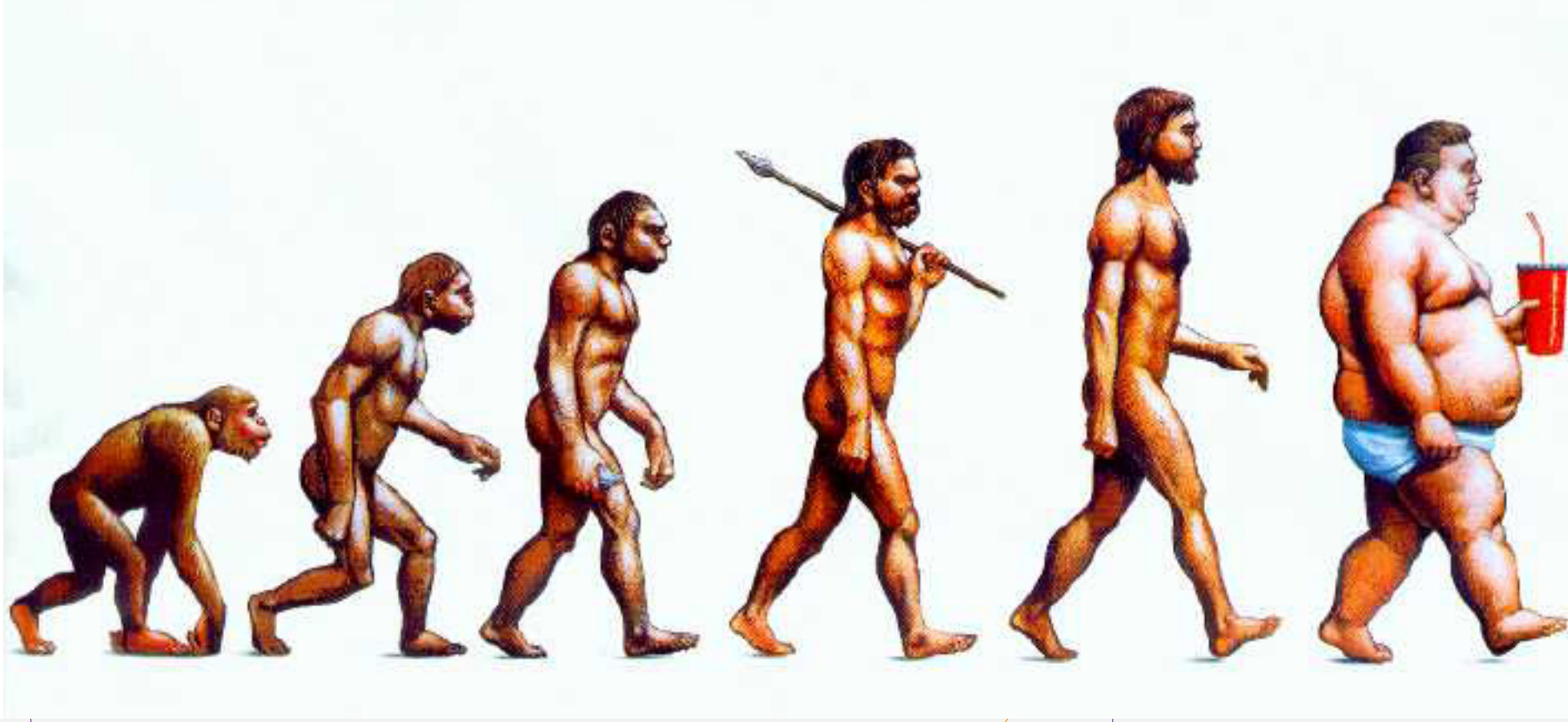
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The Last 100 Years



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There are many, many triggers that contribute to the end result of advanced inflammatory diseases. Foods is one of the most important.

But arguably, not the most important



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Stress is a Central Player in Functional Alterations of the Hypothalamic-Pituitary-Adrenal-Thyroid-Gonadal Axis



The adrenals are the first combination that we should unlock as they are the gateway to this steroidogenic balance.

Adrenal

↓

Thyroid

↓

Sex Steroids



Iodine

Halogens are a group of elements in the periodic table, which include iodine, chlorine, bromine, fluorine and astatine. Molecules of thyroid hormones contain iodine in their structure.

The trouble is that other halogens, such as fluorine, chlorine and bromine have an ability to push iodine out of those molecules, making them dysfunctional and causing hypothyroidism.



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FEMS Microbiology *Letters*, 363, 2016, fnw005

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FEMS Microbiology Letters, 363, 2016, fnw005

doi: 10.1093/femsle/fnw005
Advance Access Publication Date: 12 January 2016
Research Letter

RESEARCH LETTER – Professional Development

Comparing the temporal colonization and microbial diversity of showerhead biofilms in Hawai'i and Colorado

Combined with the likely possibility that pressurized running water sloughs and aerosolizes microbe-containing biofilms, there is the potential to inhale bacteria-laden water droplets. This notion is supported by the finding that shower mist contains *L. pnuemophila* and *P. aeruginosa*

ABSTRACT

The household is a potential source of opportunistic pathogens to humans, a particularly critical issue for immunodeficient individuals. An important human–microbe interface is the biofilm that develops on showerhead surfaces. Once microbe-laden biofilms become aerosolized, they can potentially be inhaled into the lungs. Understanding how quickly a new showerhead becomes colonized would provide useful information to minimize exposure to potentially pathogenic environmental microbes. High school scientists sampled the inner surfaces of pre-existing and newly fitted showerheads monthly over a nine-month period and applied standard microbiologic culture techniques to qualitatively assess microbial growth. Water chemistry was also monitored using commercial test strips. Sampling was performed in households on Oahu, Hawai'i and Denver, Colorado, representing warm/humid and cold/arid environments, respectively. Pre-existing showerheads in Hawai'i showed more diverse microbial growth and significantly greater microbial numbers than a comparable showerhead from Colorado. New, chrome-plated or plastic showerheads in Hawai'i showed diverse and abundant growth one month after installment compared to new showerheads from Colorado. The pH, total chlorine and water hardness levels varied significantly between the Hawai'i and Colorado samples. Enthusiastic student and teacher participation allowed us to answer long-standing questions regarding the temporal colonization of microbial biofilms on pre-existing and new showerhead surfaces.

Keywords: microbial biofilms; environmental health; water quality; citizen science; microbiology education

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MAJOR ARTICLE

Mycobacterium tuberculosis Invasion and Traversal across an In Vitro Human Blood-Brain Barrier as a Pathogenic Mechanism for Central Nervous System Tuberculosis

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Background. Central nervous system (CNS) tuberculosis is a serious, often fatal disease that disproportionately affects young children. It is thought to develop when *Mycobacterium tuberculosis* breaches the blood-brain barrier

M. tuberculosis was found to both invade and traverse the model BBB significantly more than did *M. smegmatis* (a nonpathogenic mycobacterium). Invasion by *M. tuberculosis* across the BBB required host-cell actin cytoskeletal rearrangements.

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development of parenchymal (cortical) and meningeal tuberculomas [8–13]. Tuberculous meningitis may further develop when mycobacteria are released into the subarachnoid space because of caseation of these tuberculomas [9, 14]. It is clear that *M. tuberculosis* invades the CNS, but the host and microbial mechanism(s) involved in this process are poorly understood. Theoretically, *M. tuberculosis* may cross the BBB as free (extracellular) organisms or via infected monocytes/neutrophils. Although the latter hypothesis seems attractive, such cellular traffic is severely restricted into

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Opportunistic pathogens enriched in showerhead biofilms

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The environments we humans encounter daily are sources of exposure to diverse microbial communities, some of potential concern to human health. In this study, we used culture-independent technology to investigate the microbial composition of biofilms inside showerheads as ecological assemblages in the human indoor environment. Showers are an important interface for human interaction with microbes through inhalation of aerosols, and showerhead waters have been implicated in disease. Although opportunistic pathogens commonly are cultured from shower

actual numbers and diversity, because most microbes are not readily cultured with standard methods (13, 14). Consequently, we used culture-independent methodology based on ribosomal RNA gene sequences to identify the composition of assemblages of microbes associated with showerhead surfaces over a wide geographical area of the U.S. Many of these microbes are closely related to organisms common in water, but some microbes of potential public health concern are enriched to high levels by the showerhead environment.

Particularly striking was the finding that sequences representative of non-tuberculous mycobacteria (NTM) and other opportunistic human pathogens are enriched to high levels in many showerhead biofilms, >100-fold above background water contents.

dark, and frequently replenished with low-level nutrient resources and seed organisms. Biofilms form on interior showerhead surfaces and potentially expose the user to a cohort of unknown, aerosolized microorganisms. Shower aerosol particles can be sufficiently small to carry bacteria deep into the airways (1). Pulmonary disease and other health risks such as asthma, bronchitis, and hypersensitivity pneumonitis are associated with inhalation of both viable bacteria and inviable microorganisms or their components (2–4). It has been hypothesized that the rise in pulmonary infections by nontuberculous mycobacteria (NTM) over recent decades is linked to increased use of showers rather than baths (5). Immune-compromised populations are on the rise; thus, identification of anthropogenic reservoirs of potential pathogens is of public health concern (3, 6).

Previous microbiological studies of showerhead biofilms have used culture methodology to detect and identify microbes, and have focused primarily on *Legionella pneumophilia* (7, 8, 9) and *Mycobacterium avium* (10–12). These organisms commonly occur in municipal waters and several studies have traced both *L. pneumophilia* and *M. avium* infections in hospitalized patients to microbes in their home showers (9, 10, 12).

Despite implication as a potential source of disease, the microbial composition of the showerhead environment is poorly known. Characterization of natural microbial communities by use of culture techniques may drastically under-sample the

Composition of Showerhead Communities. Ribosomal RNA gene sequences from natural microbial communities seldom are identical to previously encountered sequences. To relate these environmental sequences to named organisms, we binned the sequences into operational taxonomic units (OTUs) of greater than or equal to 97% identity, which corresponds approximately to the rRNA gene sequence variation seen in studied microbial species (15). Most of the sequences fell into species-level (≥97% identity) or genus-level (≥95% identity) bins with one or more named representative.

Fig. 2 summarizes the distribution of genera that comprised at least 0.5% of the total showerhead clones sequenced, grouped by municipality of origin (Dataset S1 shows all observed sequence types). The showerhead communities were comprised of multiple organisms, and the specific organisms varied from site to site. In

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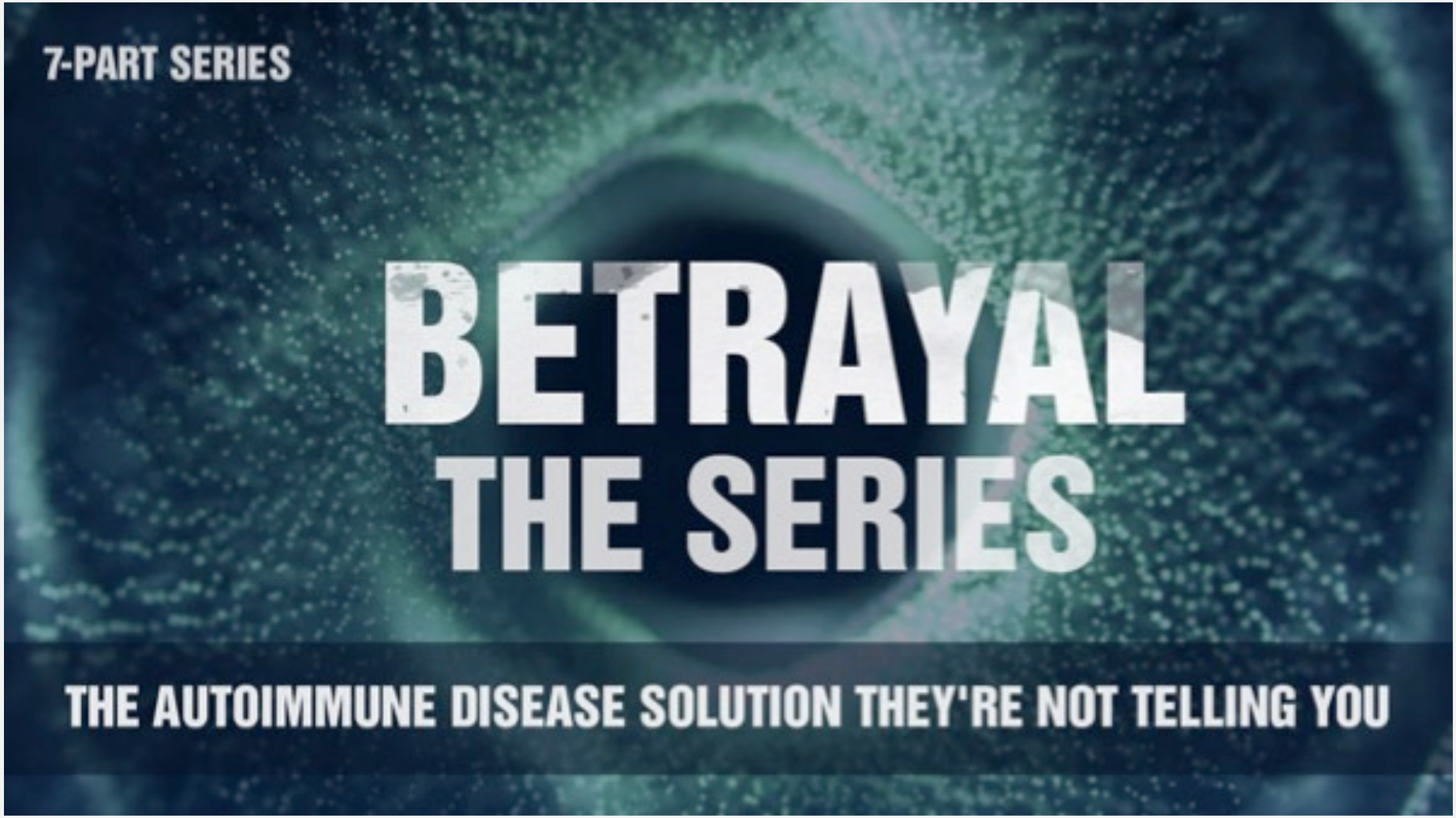
The authors declare no conflict of interest.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. EU629353–EU635442 and EU697021–EU697072).

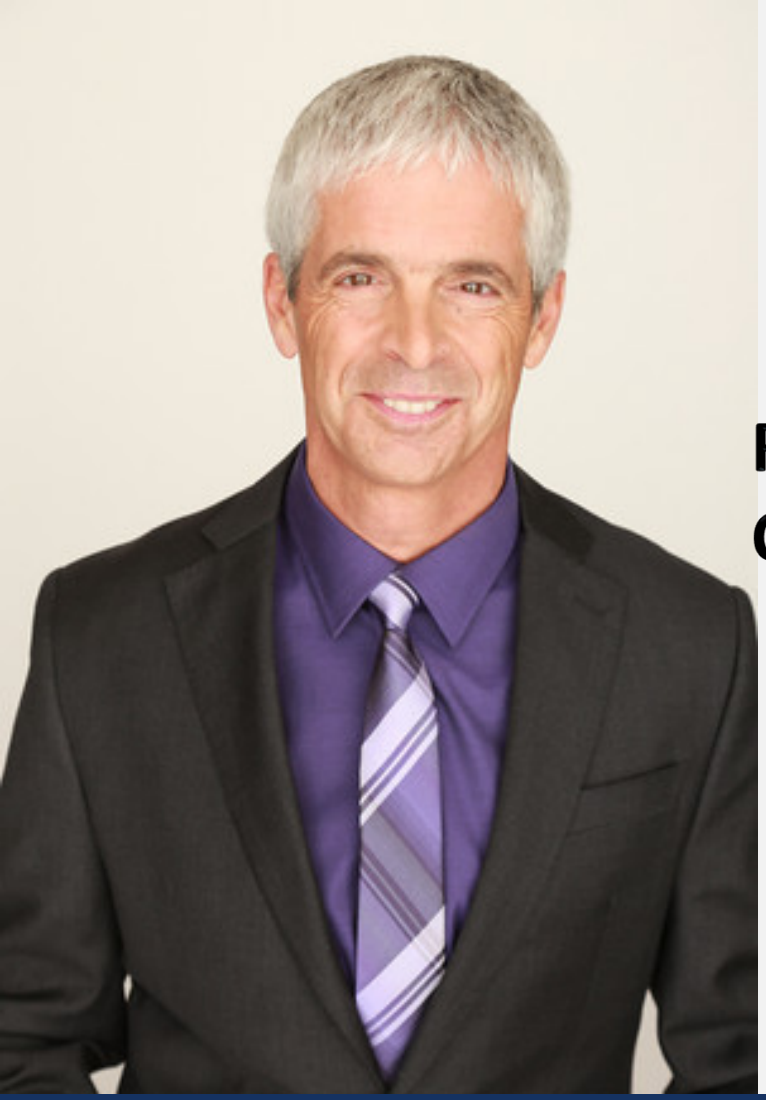
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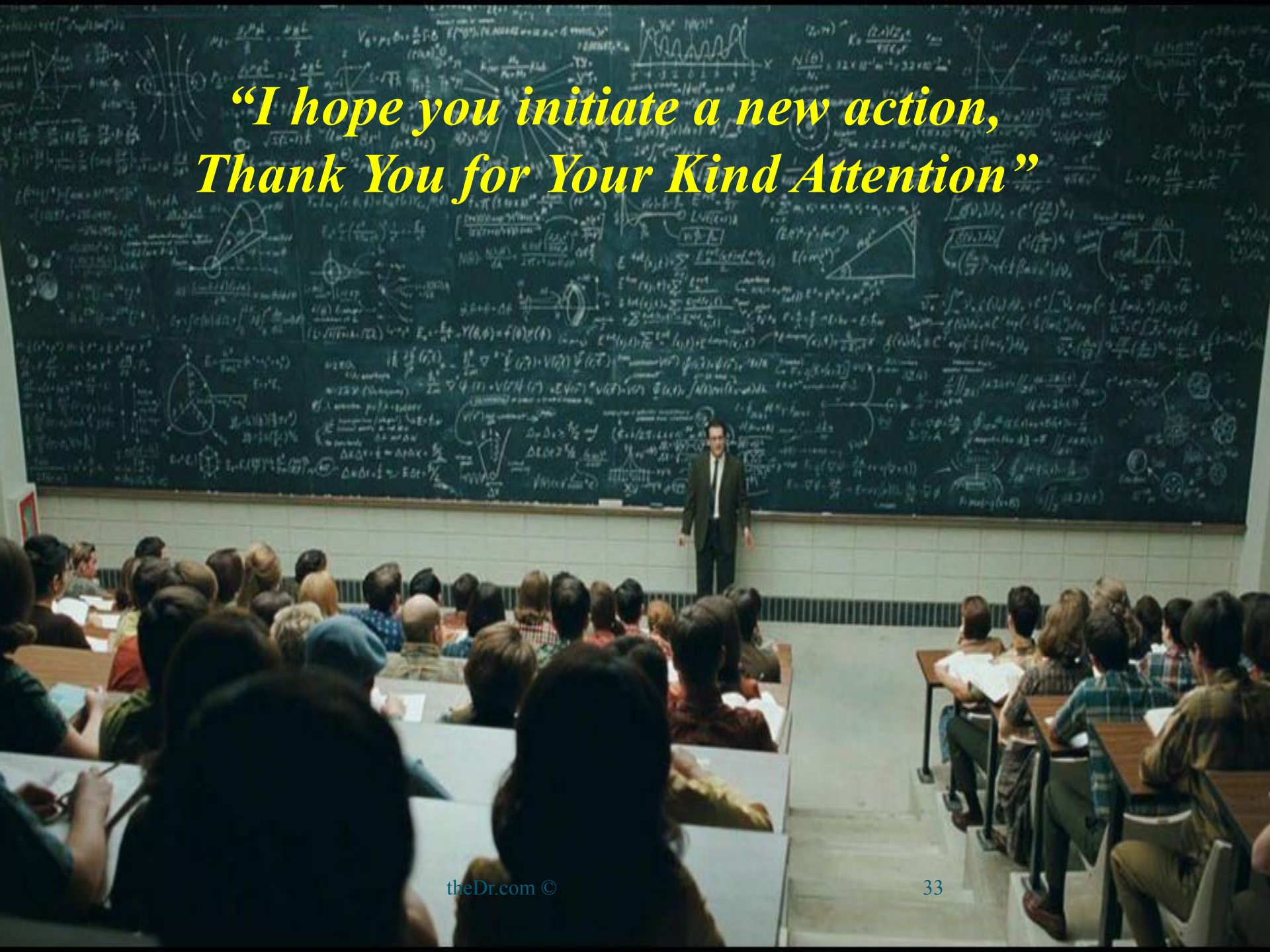
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