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RESEARCH ARTICLE

TUBB1, TUBA4A and MAPK as Indicators of Die-Back Degenerative Central Nervous System Disease in Patients Sickened by Specific Exposure to the Interior Environment of Water- Damaged Buildings

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ABSTRACT

Beginning with Possible Estuarine Associated Syndrome (PEAS) in 1998 and followed by numerous studies on similar types of exposures, patients with illness associated with biotoxin exposure routinely have had symptom rosters dominated by executive cognitive dysfunction, including recent memory deficits, difficulty in concentration, difficulty with word finding, decreased assimilation of new knowledge, confusion and disorientation, tremors, headaches, vertigo, and unusual pains. In addition, tremors, headaches, vertigo, unusual pains and metallic taste. Toxins involved variously have included those found in toxin-forming dinoflagellates, including Pfiesteria and ciguatera; cyanobacteria, including Microcystis, Cylindrospermopsis and Lyngbya wollei; post Lyme syndrome and Babesia; as well as organisms found in damp buildings, including Aspergillus versicolor and A. penicillioides, Stachybotrys chartarum, Chaetomium globosum, Wallemia sebi, Actinobacteria species, especially Corynebacteria tuberculostearicum and Propionibacterium acnes; as well as bacterial endotoxins and beta-glucans. These illnesses have been called chronic inflammatory response syndromes (CIRS).

The mechanism of neurologic findings has remained elusive despite studies showing successful treatment with intranasal vasoactive intestinal polypeptide (VIP). Neurocognitive testing has only been performed in PEAS patients, showing profound deficits in learning and higher cognitive functioning. These CIRS patients have had brain imaging without consistent findings, including MRI, EEG, and CT of the brain. NeuroQuant has shown findings that fit a "fingerprint" found in patients with specific causation and confirmed exposure to Actinobacteria and endotoxins. Fungal exposure shows disproportionate enlargement due to interstitial edema in the forebrain parenchyma and cortical grey, with a diminished caudate nucleus size. These findings have not been found in controls. The recent inclusion of transcriptomic studies using GENIE has confirmed that specific causation can be identified for Actinobacteria (48% of total confirmed cases), bacterial endotoxins (28%) and fungi (7%) in CIRS patients. The combination of NeuroQuant and GENIE has implicated excessive production of cytoskeletal tubulin genes TUBA4A and TUBB1 as risk factors for specific fingerprints for die-back degenerative central nervous system (CNS) injury in patients with illnesses associated variously with exposure to Actinobacteria, fungi and endotoxins.

This study seeks to implicate a causal abnormality of excessive expression of tubulin genes TUBA4A and TUBB1. Given the role of these genes in die-back CNS degenerative diseases, such as Alzheimer's, amyotrophic lateral sclerosis and Parkinson's disease, and anecdotal successful treatment of CIRS patients with elevated TUBA4A and TUBB1, we suggest the possibility of treatment of tubulin excess may have a role in clinical improvement seen in die-back CNS degenerative diseases. Elevated levels of MAPK are also risk factors when combined with elevated levels of TUBA4A and TUBBI.

ACRONYMS

ALS	amyotrophic lateral sclerosis
CNS	central nervous system
CIRS	chronic inflammatory response syndrome
GENIE	Genomic Expression: Inflammation Explained
MAPK	mitogen-associated protein kinase
PEAS	Possible Estuarine Associated Syndrome
TUBB1	Gene coding for protein tubulin beta-1
TUBA4A	cytoskeleton microtubule gene
VIP	vasoactive intestinal polypeptide
WDB	water-damaged buildings

Introduction

Pfiesteria was found in 1997 to be causative of a distinctive illness in exposed patients recreating or living along stretches of the Pocomoke River in Maryland in which fish were killed, and humans were severely affected following exposure to the toxin produced by this dinoflagellate. Similar illnesses that meet all criteria for a distinct biotoxin illness syndrome have been recognized[1]. Symptom rosters of patients with diverse causes of biotoxin illness have shown a consistent abnormality in neurocognitive and neurologic symptoms in cases separate from respiratory, gastrointestinal, and musculoskeletal (see Table 1) symptoms[2, 3]. The mechanism of injury to the central and peripheral nervous systems caused by exposure to Pfiesteria is still unclear. There is no question that executive cognitive functions were involved, as shown by a landmark paper published in the Lancet in 1998[3]. While most patients with acute Pfiesteria Human Illness (called PEAS by the CDC) showed either self-healing or improvement in response to an orally administrated anion binder, cholestyramine, a drug normally labelled for human use to lower cholesterol, some patients have persistent neurocognitive impairment. These individuals were studied and were found to have abnormalities in a CNS volumetric program, NeuroQuant, labelled by the FDA for use in 2007. Moreover, abnormalities were seen in magnetic resonance imaging, with elevated levels of lactate identified not found in controls (Shoemaker, 2013, unpublished).

The medications used to treat chronic biotoxin-associated illnesses, including Pfiesteria, became more sophisticated, and sequential improvement with the use of medications underlaid the publication of a treatment protocol in use today[4].

TABLE 1 NEUROLOGIC SYMPTOMS

	Controls	Cyano	WDB-1	WDB-2	PEAS	Ciguatera	Lyme
N=	239	10	156	288	42	100	352
Unusual Pains	<5	50	62	51	.	82	86
Headache	9	90	78	66	73	78	88
Blurred Vision	<5	40	61	56	.	53	66
Memory	<5	80	83	66	84	81	80
Concentration	<5	70	81	62	35	83	82
Confusion	<5	40	75	57	24	66	72
Word Finding	<5	80	81	66	.	80	84
Assimilation	<5	80	72	65	.	78	88
Disorientation	<5	30	51	40	.	28	33

Numbness	<5	40	48	44	.	74	66
Tingling	<5	40	61	51	.	78	71
Vertigo	<5	40	39	48	16	29	37
Metallic Taste	<5	40	45	36	.	46	38

DATA TAKEN FROM CIRS CONSENSUS 2018 REF 10

Much more common sources of CIRS have been recognized, especially in patients who were exposed to the interior environment of water-damaged buildings (WDB), as well as some with Post Lyme Syndrome or ciguatera, who showed a consistent roster of neurologic symptoms representing inflammation and metabolic dysfunction in the central nervous system[5, 6,7]. Despite the availability of a peer-reviewed, published protocol for treatment, it was not until vasoactive intestinal polypeptide (VIP) was in wide use that we could see the correction of multinuclear grey matter atrophy with successful treatment with VIP resolving the multinuclear abnormalities[8]. Despite these advances in the central nervous system findings with treatment, we still were left with the need to explain the pathophysiology of the central nervous system abnormalities.

A breakthrough link occurred when NeuroQuant was used to show a specific fingerprint of injury following exposure to Actinobacteria and endotoxins[9]. With a more precise definition of what was wrong with the central nervous system in CIRS cases came the unveiling of abnormalities seen in a transcriptomic program called GENIE, which began in 2015. By 2023, GENIE was able to show specific causation for patients exposed to toxigenic fungi, Actinobacteria and endotoxins. Specifically, a retrospective study revealed a significant increase in TUBA4A and TUBB1, both in endotoxin and fungal cases, plus in Actinobacteria cases[9].
This finding was based on a retrospective review of CIRS cases for which GENIE and NeuroQuant were performed looking at atrophic nuclei. In all cases of CIRS WDB, mean grey matter nuclear atrophy was 2.4/6 abnormalities, adjusted for age, found in toto for the hippocampus, amygdala, caudate, putamen, pallidum and thalamus. For Post-Lyme cases, mean atrophic nuclei were 3.0/6, with MARCoNS insensitive or resistant to vancomycin or gentamicin totaling 4.15/6. The elevated presence of TUBA4A or TUBB1 exceeded all other subsets with a mean of 5.1/6 identified atrophic grey matter nuclei. SEE TABLE 2

TABLE 2

Mean number of atrophic grey matter nuclei out of six structures

Control	CIRS-WDB	CIRS-PLS	MARCoNS	TUBA4A
			VI/VR: GI/GR	
0.9	2.4	3.0	4.15	5.1

TUBA4A and ALS: The role of TUBA4A is prominent in the literature regarding a die-back CNS degenerative disease, amyotrophic lateral sclerosis (ALS), with emphasis on mutations and failure of axonal delivery of ions and nutrients leading to die-back degenerative changes in motor neurons. Familial ALS is associated with mutations in TUBA4A[11]. Another ALS-regulated protein is FUS protein[12]. HDAC6 regulates mutant SID1 through aggregation and tubulin acetylation[13]. HDAC6 inhibition reverses axonal transport deficits in motor neurons derived from FUS-ALS patients[14].

A much greater emphasis is on the axonal cytoskeletal microtubules in ALS patients. In 2004, Jablonka focused on axonal defects of a mouse model of ALS[15]. Earlier work noted the defects in Golgi body fragmentation[16], with modification of microtubule proteins in ALS occurring before detectable ultrastructural changes were recorded and reported[17]. This paper was followed up in 2003 by another treatise on changes in microtubule-associated proteins occurring before disease onset[18]. As time passed, the ALS literature focused on microtubules and the Golgi body[19, 20, 21], concluding that microtubules form important cytoskeletal structures that form a role in establishing and maintaining neuronal polarity, regulating neuronal morphology, transporting cargo, and scaffolding signaling molecules to form signaling hubs[21]. By 2023, TUBA4A mutations were shown to define mechanisms of microtubule disintegration in ALS[22]. The authors state, "The importance of microtubules for cellular long axon infrastructure and the function of motor neurons underscores the central role of cytoskeleton dysfunction as a possible mechanism of ALS. TUBA4A is one of two major tubulins of microtubules, suggesting cytoskeleton changes are linked to a broad spectrum of human neurological diseases referred to as "tubulinopathies."

Microtubule research expanded to other CNS degenerative entities, including progressive motor neuropathy[23], BMAA neurotoxicity[24] and proteinopathy[25]. We feel that the potential for tubulinopathies and TUBA4A to have relevance in discussing axonal die-back in CIRS and CNS.

Methods:

We used a retrospective study design using a deidentified database of transcriptomics findings that report results of gene activity compared to controls from a library of 210 genes in 1822 patients. We extracted cases that showed positivity of tubulins TUBA4A and TUBB1. We used the same protocol for reporting gene activation as reported previously[9]. We further defined the data extraction to compare tubulin cases with specific causation by endotoxins (CD14, TLR 2, TLR4), by Actinobacteria (TGFBR 1,2,3 and one or more of six MAPK genes) and by fungi (two or more caspase genes involved with apoptosis and three or more of six MAPK genes)[9]. We then assessed the incidence of tubulins by sorting by elements that supported specific causation, as this step defined true cases of CIRS-WDB. We looked for correlation of tubulins in the subsets of known causative conditions of CIRS-WDB. Using the correlation, we could assess the potential for CNS causation of illness with findings reported for reported types of die-back degenerative disease.

Patient records were reviewed independently for MAPK numbers with our library consisting

of MAP2K3, MAP3K5, MAPK1, MAPK14, MAPK3 and MAPK9. Sixty-two patients had one MAPK positive gene; 37 had two MAPK genes positive; 30 patients had three MAPK genes positive; 19 had four MAPK genes positive; 8 had upregulation of five MAPK genes, and one had all six MAPK gene upregulations.

We looked at incidence of cases with specific causation as a percentage of tubulins, understanding that a case might have specific causation from more than one category of exposures.

Results:

386 patients were positive for TUBA4A. Correlation with endotoxin genes was made, finding 128 showing positive TLR2 gene upregulation, 139 showing positive TLR4 gene upregulation, and 114 showing positive CD14 gene upregulation. The correlation of TUBA4A positivity with TL2, TL4 or CD14 was found in 98.7% of cases.

Charts were screened for MAPK positives with positivity of TGFBR 1, TGFB2, AND TGFBR3 as these findings identify specific causation for Actinobacteria. We sorted by TUBA4A positivity, with 70 having one MAPK gene positive, 64 having two MAPK genes positive; 68 had three MAPK genes positive; 51 had four MAPK genes positive; 7 had five MAPK genes positive, and none had six MAPK genes upregulated. These findings in 245 patients support specific causation for Actinobacteria in 67.3% of cases with positive tubulins. Note that the screening was focused on a subset of genes positive for TUBA4A and TUBB1 stratified by their known complicating factors or gene correlation with TL2, TL4 and CD14 as a group or MAPK as a group.

Eight genes showed positive apoptosis with two or more gene upregulations found in CASP 10, CASP 3 and/or CASP 8. Fungal specific causation amounted to 2.1% of TUBA4A cases.

For TUBB1, out of the 386 patients, 202 had TUBB1 gene upregulation; 72 were positive for TLR2 upregulation; 59 were positive for TLR4 and CD14 gene upregulation was 71, showing 52.3% of cases carry endotoxin-associated genes.

Five cases of TUBB1 showed apoptosis-positive for 2/3 of CASP 10, CASP 3, and CASP 8, totaling 1.2%.

Patients with TUBB1with one MAPK were 27% of the cases, those with two MAPK were 13%, and those with three MAPK were 11%.. The total cases with TUBB1 and MAPK positivity totaled 157, or 77.7%.

Discussion:

We have shown that TUBA4A is associated with grey matter nuclear atrophy seen on NeuroQuant in combination with exposure to WDB. We have demonstrated the presence of endotoxins, and Actinobacteria found in WDB caused chronic inflammatory response syndrome, CIRS, an inflammatory illness. The use of GENIE with NeuroQuant has offered patterns of brain injury acquired following exposure to specific toxigenic factors, including endotoxins and Actinobacteria. In this paper, we have shown that TUBA4A and TUBB1 are associated with a preponderance of genes that provide the basis of specific causation by specific microbial elements.

We have previously shown the ability of a specific peptide, VIP, to correct other sources of degenerative CNS disease. Additional

treatment efforts are underway.to indicate the resolution of elevated levels of cytoskeleton genes, as shown by GENIE, marching in step with the resolution of brain injury, as shown by NeuroQuant. There is a small, but growing literature on die-back CNS diseases, including Alzheimer's disease and Parkinson's disease [26, 27, 28, 29].

The basis to postulate causation for tubulins alone in die-back CNS degenerative disease will await prospective study confirming new onset of degeneration following confirmed exposure. The data reported here are obtained through retrospective chart review of one large data set that took three years to create. These data permit association of tubulins with specific abnormalities reflecting specific exposure to specific microbes found to reside in WDB. It is unlikely that exposure to a WDB could be studied prospectively, however, as public health measures would preclude continuing exposures to a known WDB. Given the strength of association of TUBA4A with endotoxin-associated brain injury in environmental exposure, for example, continued prospective exposure would not be ethical. The acquisition of neurological illness symptoms must by itself bring about study for associated lab abnormalities, followed by GENIE testing before CNS injury becomes evident.

Conclusions:

Brain injury occurs following multiple insults and injuries. One such pathway involves cytoskeletal genes TUBA4A and TUBB1 damaging axonal microtubules, reducing the flow of ions and nutrients to neuronal synapses, creating die-back degenerative disease. The efficacy of VIP in the correction of CIRS has been established. We look forward to the investigation of links of TUBA4A and TUBB1 to treatment of other die-back degenerative disease, understanding that correlation in the absence of specific causation is promising but not secured. The lessons learned from unveiling the association of tubulins and die-back brain injury may have relevance studying other sources of degenerative disease in which environmental causation is not apparent.

Conflict of Interest Statement:

None

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