To the reader: this is an early draft* of Chapter 16 from

Genetic and Environmental Causes of Autism

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Chapter 16. The Logic of Prevention in Autism and Gene Informed Treatment

Too much time and energy and expense has been spent in the back-and-forth between those who would like the public to continue to believe there are no links between vaccine and autism and those who acknowledge vaccine-induced immunoneurotoxicity. It's time to move on: the mechanisms are established, the science is clear: aluminum and mercury can cause autism in some people.

The question is, what are we going to do about it? There is much work to be done.

We need to build reliable risk prediction models that can inform doctors which patients should forgo exposure. These need to be researched using state-of-the-art practices for phased risk and biomarker development. We need to work out rational medical exemptions. I believe parents concerned vaccine-induced immunoneurotoxicity should be granted exemptions without any problem for their pediatricians. Pediatricians are sometime reluctant to issue medical exemptions for vaccines for fear of sanctions – primarily due to loss of income from vaccines, according to one pediatrician interviewed who asked to remain anonymous. I don't see why doctors are not pushing – hard – for autism risk models that include vaccines. Perhaps most do not do their homework on the issue, and rely on the CDC and the American Pediatriac Association's positions. I believe I can hope that at least one of those positions can be changed. I am calling on these and other organizations to to change their tune, ever so slightly – to simply inform the public that some people may be susceptible to autism from vaccines. And I urge them join the growing number of doctors and scientists who are making and demanding reform in vaccine science. This would be the most significant step toward prevention.

For those who remain skeptical, population-based retrospective studies showing no association do not meet the standards required nor level of evidence needed to enact public health policies. The vaccine schedule is untested. Genetic-informed, risk-informed association studies would be a massive improvement. Rather than use genetic and demographic factors as confounders, and removing patients in an ad-hoc, semi-block design that uses some variables as covariates, and others as exclusion criteria, let's do studies that seek to determine whether biomarkers and other risk factors exist that could have predicted which patients would have developed autism from vaccines. Let's enrich samples for people with mutations in specific genes to see if their groupwise risk of ASD in the genetically at risk minority is increased by vaccines. Let's do total health outcomes awareness studies in which all health outcomes, not just autism, are examined. And most of all, let's stop pretending the we're doing science when we use proxy measures of total exposure, rather than cumulative dose. Let's allow lifetime exposure measures to be an independent variable in both association studies and in outcome studies.

Prevention

First, we need to freeze the number of vaccines in the schedule and remove aluminum. Animal safety studies of the entire pediatric schedule should be conducted with animals with various combinations of genetic predisposition alleles. We need to reduce exposure to glyphosate, to PDBE's, and other environmental toxins and may need to consider familial risk factors during pre-natal care (e.g., ultrasound) and peri-natal care (e.g., extended umbilical cord blocking).

Until we can determine the safe levels of numbers and frequencies of vaccines in terms of antigenic-induced immunoneurotoxicity, leading to CMA, it would be unconscionable to add more vaccines. Removal of aluminum would, I think, be the more responsible of the two options. However, there should not be an unending number of vaccines added to the schedule. Mandatory vaccine laws should require renewal when the vaccine schedule changes, and should require the highest possible level of clinical evidence of long-term safety available. That means randomized prospective clinical trials. We should not pass laws that give governments power to dose the population with ever increasing vaccines, antigens, adjuvants or additives; that would be an uncontrolled experiment run amuck.

There is also good reason to remove specific vaccines from the schedule, for certain people to reduce their personal risk of chronic microglial activation. Pragmatic policies and practices are needed that reduce total lifetime exposure. This would include genetic screening using a panel of validated risk markers.

Some actions can be taken to reduce autism risk in spite of exposure. Expectant and breastfeedingh mothers with MTHFR and COMT variants should avoid folic acid, a synthetic form of folate. Such mothers can reduce autism risk by avoiding folic-acid containing pre-natal vitamins (Schmidt et al., 2011). This is only necessary for mothers with most MTHFR mutations. Carriers of some mutation, however, appear to require extra folate. Choline supplementation may help individuals with MeCP LoF mutations or deletion (Ricceri et al., 2011).

Individuals (autistics and neurotypicals) with specific variants of the HLA-DQA1 and HLA-DQB1 genes have celiac disease; their mutations cause a sensitivity to gluten and gluten-induced enteropathy (aka celiac disease). The frequency of these mutations is roughly the same across the populations, and therefore the occurrence of gluten sensitivity might be thought of as being independent of ASD.

Reducing exposure to environmental sources of excitoxic chemicals is a reasonable step for high-risk individuals as well. Faber et al. (2015) studied the effect of a cleanroom sleeping environmenta on markers of oxidative stress, immune regulation and behavior in children with ASD and found that younger children showed decreased immune dysregulation (decreased CD3 and CD4), improved behavior. Olderchildren displayed a worsening in behavior, which the authors surmised may have been to effects of a higher load of toxins released. Five children showed decreased concentrations of oxidized glutathione.

While this was an small, uncontrolled study, it shows the potential of reducing chronic and cumulative exposure. Anecedotally, one set of twins showed divergent patterns of behavior improvement that tracked their differences in 4 of 5 markers of oxidative stress.

In the meantime, a number of compounds and foods may prevent the onset of microglial activation. First, glutathione is essential. Well-meaning parents and doctors who in the past wished to use Acetominophen to reduce risk of fever after vaccination may have done more harm than good because Acetominophen depletes glutathione.

Avoiding Fevers in Pregnancy

Fevers in pregnancy should be avoided. Interactions between acetaminophen use and vaccines have not been fully studied.

To protect pregnant women from influenza, a virus-like particle vaccine is needed that does not require preservatives or adjuvants such as thimerosal and aluminum.

Risk Prediction Modeling

Understanding the etiology of autism means comprehending routes to prevention. However, autism is highly heterogeneous in its etiology, and therefore some clever and sophisticated approaches are needed that can score infants, perhaps in the pre-term, for risk of developing ASD.

The ability to perform rapid risk modeling would be valuable to determine which patients patients might fall into high risk categories to consider prevenation programs. Such individuals should be monitored for elevated glutamate in their CNS, and perhaps scanned for chronic microglial activation. Both conditions should be treated in as tailored a manner as genetic testing allows.

Known Risk Factors

A great deal of etiological factors are known, and some are reflected in the specific manifestation of ASD and in some of the common co-morbid conditions. From Bradstreet et al, (2010):

"Newer evidence, however, reveals that ASDs are associated with: oxidative stress; decreased methylation capacity; limited production of glutathione; mitochondrial dysfunction; intestinal dysbiosis; increased toxic metal burden; immune dysregulation, characterized by a unique inflammatory bowel disease and immune activation of neuroglial cells; and ongoing brain hypoperfusion."

Each of these conditions, or processes, have a root cause; identifying the root cause will alleviate the condition, and its role in ASD. Sometimes targeted treatmens will be aligned with the root cause; those treatment that are will be called 'cures'. Other treatments that do not address root causes will be considered palliative and, in the long run, ineffective. Bradstreet et al. (2010) provide a comprehensive report on which invidual biomarkers reflect physiological conditions in ASD and ADHD, and how they can be corrected medically.

We can also systematically organize the information from all of these disparate sources and derive risk models that will help parents at once reduce risk and improve the symptoms of ASD in their children.

(1) Familial Risk Markers

Recurrence risk family is between 4-13% if the first child affected is female and about 7-20% if a male. The recurrence rate increases to 25-30% if a second child is also diagnosed with ASD. Studies have shown that among identical twins, if one child has ASD, then the other has a 60 to 95% chance of being affected. (Dhillon et al., 2011).

(2) Parental Age Markers

A metanalysis by Hultmann (2011) found that men aged 40 to 49 were 1.4 times more likely to have children with a diagnosis of autism than a comparison group (15-29-year-old males). Risk of autism increased with father's age: 50-54 year old men were 2.2 times more likely to have autistic children; above 55, then risk of 4.4 times the comparison group.

One of the potential confounders for this finding is that men who tend to have children at an older age may take longer to adjust socially to adulthood, and tend to pass those genes forward to their children at an advance age. Here again we see the limits of retrospective epidemiological studies. This would an example in which the group of interest (older men who have children later) actually represented a cryptic genetic subgroup.

Even so, advanced parental age could be a proxy variable for a specific causal factor; one would have to study whether parental age adds additional, independent information to a prediction model beyondother variables, such as genetics and cumulative environmental exposures.

Other studies have shown that young fathers have similar risks to older fathers. Is this methylation in action? Or share environment? It could possible be a cohort effect. Or is there genetic variation (e.g., for risk taking) that can lead to teen fathers that is also associated with ASD risk? Research is needed to tease these factor apart.

(3) Genetic Variation Markers

In addition to numbers of CNV in general, a condensed panel of genetic markers might be one route to assaying autism risk, however, this could miss important de novo variations. A specific approach to autism risk whole genome sequencing is needed that guarantee clinical-level accuracy of all types of genetic variation without bias and with extremely low false positive rates.

(4) Biomarkers of and History of Exposures

A checklist of known exposures – including cumulative doses and mercury and aluminum in vaccines – is needed. Treatment with acetaminophen at an early age is a risk factor. Some of these will be difficult to assess, and thorough environmental exposure surveys can be expensive. Development opportunities and need exists for standard assays for macrophage measures of aluminum, mercury and other toxins, which may be most informative in general, and should be given highest priority as high measures would indicate course corrections to reduce exposures.

(5) Metabolic, Physiologic, Cellular Biomarkers Indicating Risk

More readily available markers exist within patients themselves – antibrain and specific Ig antibodies in the mother, and child; glutathione levels, glutamate levels in the CNS, GFAP measures of microglial activity, blood cytokine levels, oxidatative stress and mitochondrial dysfunction biomarkers, metabolic markers of detoxification pathways,neurotropic markers , vasopressin, serotonin level, skin conductance measures, measures of brain levels of connectivity are all potential candidates that might be used in a screening program to inform parents on specific treatment routes likely to be relevant for a patient, and the need for course corrections including dietary change and vitamin D3 deficiency. Serotonin has been

studied in detail as a biomarker and shows promise (Pagan et al., 2014). The expression of the genes neurotrophin 3 and 4 and a neurotrophin receptor (P75) was found to be potential biomarkers of ASD in the blood (Segura et al., 2015). Nelson et al. (2001) also reported finding neuropeptides and neurotrophins in neonatal blood of children with autism and mental retardation.

Many conditions share symptoms with autism – and many appear to share molecular etiologies. As we will see, the rich information available to patients via genetics is likely much more important to individual patients and families in defining roadmaps for specific action in terms of prevention, therapies and treatments, and specific definitions of prove to be extremely important in clinical research. A lifelong neurological disorder screen given at annual check-ups is therefore much more likely to prove cost-effect than merely a screen for autism.

Medical Exemptions and Medical Rights Exemptions from Vaccines

Prevention includes medical exemptions from vaccines for those concerned. The number one side effect of vaccines is fever. Children with autism are likely to experience recurrent immunological hyperactivation. Siblings of children with autism may be at more risk, due to the familial risk and to birth order. Thus, it is reasonable to propose medical exemption in light of proven risk of adverse events related to vaccine, especially if the parents report temporal association. Women with anti-brain antibodies in their blood should certainly avowing having their immune systems activated intentionally during pregnancy. And genetic testing is available to assess biologicallyinformed risk.

If a woman does decide to get the flu shot while pregnant, she should wait until the last trimester, and she should request the vaccine without thimerosal (*contra* CDC's recommendations).

Medical exemptions have been in place with vaccines for years; classically the wisdom was to never vaccinate the sick. Now, this wisdom appears to no longer be in place.

Human beings already have Medical Rights, under US and Internation Law that protect them for being experimented on, or for having medical procedures conducted upon them, without informed consent.

A recent spate of rather hateful action on the part of some healthcare workers is worrisome; it is illegal to use force or coercion to cause a person to submit to a medical procedure. Doctors who threaten patients with sanctions of refused health care could in principle lose their licenses for coerced medical practice, and civil suits could be filed against them for physical harm. Of course, any medical practice conducted upon any patient without consent should be considered assault and battery and should be reported to the local police.

Folic Acid During Pregnancy

Folic acid supplementation during pregnancy reduced incidence of autism (Surén et al., 2013), and has been observed to alter gene expression in genes involved in autism (Barus et al., 2015).

Iron During Pregnancy

Iron supplements taken during pregnancy also appear to reduce risk of ASD diagnosis (Schmidt et al., 2014).

Treatments

There are autism advocacy groups who spark at the idea that autism is a disease. They are sensitive to word use and do not like being considered having a disease, something to be cured. It is their position that autism/ASD is part of what makes them who they are. They do not want to be labeled 'disabled'; they want to labeled 'differently abled' and want to know that people see them just like other people: 'perfectly imperfect' (see, for example, Richard Âû's blog, 2015). And I understand why. This world can be cruel. There are mean-spirited people who will look down on those with autism or Asperberger's. And we should certainly respect the rights of people on the spectrum to have and expression their opinion.

However, some autistics cannot write blogs. They cannot speak. They cannot walk well. They cannot learn language, or math, or music. Other autistics are particularly gifted in some domains. They, like all people, are beautiful. And what science and biomedicine will do for them, and has done for them, is enabled their immune system to stop consuming their neural precursor cells and their dendrites. These parents and individual simply want to enable, and empower their child's mind, and their own mind, to grow.

As late as 2014, it was generally perceived that while co-morbid conditions that co-occur with autism may respond to treatment, the core components of ASD did not respond to treatment (Lai et al., 2014). That may not longer be the case. As treatments come forward for specific domains of expression of autism, no one will force anyone to take the treatments. It will be up the parent, or the adult themselves, to make the decision. Patients have a right to informed consent both for vaccination risks and for treatments of any kind. If an autistic person cannot make their own consent, it's up to their legal guardian to do so.

I interviewed an autistic fellow, Mr. Jonathan Mitchell, for this book who would really like to have new, effective treatments for autism. His call for cures has made national news as they were interpreted to be insulting by people in the neurodiversity movement.

Jonathan thinks it should be curable. He told me:

"Sure, I'd like to have less executive function problems, and sure, I'd like to have my hand flapping start. I'd like to be more approachable, socially, you know, and maybe have a date. You know, I've never even had a date."

Given that environment toxicity is preventable, but perhaps not reversible, cures might not be the best terms for the possible goal. It is important to keep in mind that there is difference between treating and curing autism. Treatments are aimed at specific phenotypes; cures would undo the damage caused by the toxins. The best and most effective doctors see the patient, not the disease.

Pharmaceuticals

Very few medicines development specifically to treat AD/ASD overall; antipsychotic medications may be useful for to modulate behavior problems like aggressiveness, and can appear to ameliorate repetitive behavior patterns. Seizures are managed with anticonvulsant medications, and bipolar symptoms are approached with typical treatments for the symptom.

That said, so far, population-based medicine has for the most part failed to render significant success in the treatment of autism/ASD. It's pretty clear which routes biomedical science could go to help shut down brain inflammation. In this chapter, in spite of the misgivings and with a great deal of respect for people on all sides of the 'curing autism' issue, I review past, current and future treatments of autism/ASD.

Aggression

The US Food and Drug Administration has approved the atypical antipsychotic drugs risperidone and aripiprazole are approved by for treatment of aggression and irritability in autistics aged 5-16. Risperidone can reduce aggression in mice with an R451C (arginine to cysteine residue 451 substitution) mutation in neuroligin-3 , NL3). This mutation is found in some cases of ASD (Burrows et al., 2015).The basis of approval for use in ASD is in part that it is used for the treatment of schizophrenia, which shares clinical features with ASD such as social deficits and shifts in the NMDA, GABA and dopamine receptors. The drug has been shown to alleviate repetitive behaviors in mice, but has no impact shown (thus far) for social deficits (Jeste and Geschwind, 2014).

Acetylcysteine

Authors of a case study (Stutzman and Dopheide, 2015) concluded that treatment with acetylcysteine improved ASD symptoms, including irritability and aggression, in their teenage patient.

"A 17-year-old Hispanic male with ASD and intellectual disability was hospitalized for inpatient psychiatric treatment due to impulsive and violent behavior. Despite receiving various medications in the initial weeks of hospitalization, including intramuscular lorazepam and diphenhydramine injections (four days a week on average), the patient continued to exhibit aggressive and unpredictable behaviors. Treatment with 20% acetylcysteine oral solution was initiated at a dosage of 600 mg twice daily as an adjunct to quetiapine therapy. Over the next six weeks, reductions in the patient's aggressive behavior, tantrums, and irritability were noted. The use of as-needed medications to control aggression was decreased, and the dosage of quetiapine was lowered from 700 to 400 mg daily over the course of the hospitalization. Acetylcysteine was well tolerated, with no observed or reported adverse effects. Unlike clonidine or guanfacine (other medications used for ASD-related behavioral symptoms), acetylcysteine is not sedating; moreover, it lacks the metabolic, extrapyramidal, and endocrine adverse effects of atypical antipsychotics. Published data from small controlled trials and case reports suggest that acetylcysteine use is associated with improvements in irritability and aggression in prepubertal children with ASD; these therapeutic benefits may be associated with acetylcysteine's glutamatergic, dopaminergic, antioxidant, and anti-inflammatory properties.

Leads for Treatment of Speech and Language Processing Disorder in Autism

Intranasal delivery of davunetide (NAP), a neuroprotective octapeptide NAPVSPIQ, from the ADNP protein, has been reported to alleviate cognitive abnormalities observed in a knockout mouse model (Vulih-Shultzman et al., 2007; Gozes et al., 2011).

The mechanism of action of NAP has been elucidated, and involves interaction of ADNP with the MAP1LC3 protein (autophagy regulating microtubule-associated protein 1 light chain 3) with microtubule end-binding (EB) proteins (Gozes, 2015). MAP1LC3 is one of the genes in the chromosome 16 region 16q24.2, found to be association with autism (Handrigan et al., 2013). The ability of macrophages to function properly is highly dependent on microtubules.

A number of drugs approved for the treatment of Alzheimer's disease are under exploration for use in ASD. Some target specific phenotypes; many others target microglial activation.

Social Skills

Tetrahydrobiopterin is a naturally occurring substance (essential cofactor) that targets L-dopamine production pathways. Administration of 20 mg/kg Tetrahydrobiopterin (BH4) per day led to significant improvements in social awareness, autism mannerisms, hyperactivity, and inappropriate speech (Fryet al., 2010). Placebo controlled studies in Japan showed long-term efficacy and very minor side effects (Takesada et al., 1990)

Treatment of AD with inhaled oxytocin has been observed to increase the frequency and duration of eye contact and improvement in social skills in autistic children. Auyeung et al., (2015) found in a randomized, placebo-controlled clinical trial that doses of 24 IU of oxytocin were administered to adult males with AD diagnosis, and control males enhanced gaze to the eyes in both the autism and control groups. Within the autism group, oxytocin had the largest effect on fixation duration in individuals with impaired levels of eye contact at baseline.

This was not the first study of the potential utility of oxytocin to improve social skills in autism. It had already been shown to increase retention of social cognition (Hollander et al., 2007), and clinical trials point to improvements in emotion recognition and eye gaze (Preti et al., 2014; Guastella et al., 2010). In Asperger's, oxytocin improves emotion recognition and social skills including turn-taking and a decrease in social anxiety (Andari et al., 2010). A more recent study (Yatawara, 2015) also showed that oxytocin treatment improved social interaction deficits in young children with autism. Domes et al. (2013) found that right amygdala activity to facial stimuli increased in ASD in response to treatment with oxytocin. Randomize placebo controlled fMRI studies have shown that oxytocin restores activity in the ventromedial prefrontal cortex (vmPFC) in a friend/foe recognition task-dependent manner (Aoki et al., 2015). It is generally recognized that oxytocin mutations may not represent important variant in all, or even most cases of autism. Tansey et al. (2010) found that no OXTR single nucleotide variant alleles to be significantly associated with autism in Irish, Porteguese, and British cases of autism after correction for multiple comparisons. However, the lack of a strong association of genomic variants (SNVs, CNVs, etc) is due to the heterogeneous nature of autism/ASD, and the finding does not mean that oxytocin receptor mutations are not very important in a subset of cases.

Vitamin D3

Vitamn D deficiency is known to exist in autistics. Feng et sound that serum levels of Vitamin D3 were significantly lower in autistic children compared to neurotypicals. They found that serum levels were also negatively correlated with ABC total scores and language subscale scores. Supplementation with vitamin D3 supplementation, autism symptom scores were significantly improved, and improvement seemed more significant in younger children.

Alleviating Mitochondrial Dysfunction

Studies have shown some promise for mutation-based and non-mutation based mitochondrial dysfunction, notably carnitine, co-enzyme Q10, ubiquinol and B-vitamins (Rossignol and Frye, 2012). Multiple molecular dysfunctions exist, however, and specific targeted treatments may be possible for each.

Serotonin Pathway Dysfunction

Serotonin reuptake inhibitors are used for the depression and obsessive compulsive disorder (OCD) in children 7 years and older, divalproex sodium used to treat manic symptoms and epilepsy, and the psychostimulant drug methylphenidate used to treat attention-deficit hyperactivity disorder (ADHD). Targeted therapy is now possible given that whole genome and whole exome sequencing is possible; mutations in the relevant genes and pathways may indicate likely efficacy, lack of efficacy, and/or safety issues with these drugs.

The outcomes of use of these drugs in autism/ASD have been mixed, at best, and general prescriptions are still hit-or-miss for any individual patient. Gene-guided treatments are needed so patients and parents can avoid the disappointment and attrition of will that some from ineffectual treatments, which can be replaced with experiences of joy and celebration at young patients' renewed progress in development and learning.

Redox Metabolism

Frye and Rossignal (2014) discuss the use of methylcobalamin with and without folinic acid and vitamin C and N-acetyl-l-cysteine in other studies, with improved core and associated ASD symptoms

Frye RE, Rossignol DA 2014. Treatments for biomedical abnormalities associated with autism spectrum disorder. Front Pediatr. 2:66. doi: 10.3389/fped.2014.00066.

Chronic Microglial Activation

If one focuses on the root cause of autism, however, a great number of opportunities exist to reduce microglial activation. Given that immunoneuroexcitotoxicity via chronic microglial cell activation is a prevalent, life-long root cause of autism, and that microglia activation can be prevented in a myriad of ways, rational design of a study that complementary treatments with drugs – and supplements - that

shut down microglial activation via different routes should be high priority. Otherwise, autistics will treated for symptoms, not reversal of causes, of their condition.

Chronic microglial activation is a positive feedback loop, which, if short-circuited, could alleviate symptoms in numerous domains of AD/ASD. People with ASD should understand that no one wants to take away what makes us special. The type of damage that can be stopped ranges from simple migraines and allergies to severe intellectual disability, lack of speech and language, and death.

Table 1. Examples of Possible Future Treatments By Phenotype

Given the central role of microglial hyperactivation, tiered treatment strategies might be worth exploring. Assessment of symptoms after each tier might indicate rationale, or obviation, of treatment of further symptoms. Not all of these drugs are approved on-label for treatment of autism or autism symptoms.

Microglial activation	Amantadine*, Luteolin, cannabinoids, many others*
Social	*Oxytocin, Tetrahydrobiopterin (Cofactor BH4)*
Language Delay/Loss	*adenosine receptor agonists*
Repetitive Motor	*Fluoxetine, Risperidone*
Seizures	carbamazepine, ketogenic diet
Aggression	aripiprazole
Gastrointestinal	*FMT, Vancomycin*
Renal peptiduria	*Vitamin E, Selenium*
Gene-Directed Treatment Examples	
Mitochondrial Dysfunction	*carnitine, various drugs*
SCN1A (Serotonin)	*Clonazepam (microdoses)*

Hollander E et al., 2012. A double-blind placebo-controlled trial of fluoxetine for repetitive behaviors and global severity in adult autism spectrum disorders. Am J Psychiatry. 169(3):292-9. doi: 10.1176/appi.ajp.2011.10050764.

McDougle CJ et al., 2005. Risperidone for the core symptom domains of autism: results from the study by the autism network of the research units on pediatric psychopharmacology. Am J Psychiatry. 2005 Jun;162(6):1142-8.

Tanimura Y et al., 2010. Indirect basal ganglia pathway mediation of repetitive behavior: attenuation by adenosine receptor agonists. Behav Brain Res. 2010 Jun 26;210(1):116-22. doi: 10.1016/j.bbr.2010.02.030.

Anti-inflammatory therapy designed reduce microglial activation to non- deleterious levels holds the promise of slowing and perhaps halting progressive brain injury.

Luteolin, for example, is a bioflavinoid antioxidant that reduces microglia activation. Treatment of activated microglia with luteolin blocked pro-inflammatory and pro-apoptotic gene expression. Ramification of microglia (return to resting state) was significantly increased. Activated microglia showed reduced nitrous oxide secretion (Dirscherl et al., 2010). Chamomile tea is one good source of luteolin. Theorhides et al.,2015 found that "brain fog" and inflammation were both reduced by luteloin, and Tsilioni et al., (2015) found that autistics with luteolin in their diet showed reductions in markers of microglial activation (TNF and IL-6 levels) in their serum.

Sulforaphane attenuates the LPS-induced increase of IL-1β, IL-6 and TNF-α expression in microglia Brandenburg et al. (2010). Sulfoaphane also dramatically improved social skill sin 2/3 of patients in a prospective, placebo-controlled clinical trial (Singh et al.., 2014). Forty-six percent of patients treated with Sulfoaphane exhibited noticeable improvement in social interaction, 54 percent improved in aberrant behaviors, and 42 percent improved in verbal communication.

A large number of Microglial Suppressors is reviewed in the Appendix to this chapter.

The Paradox of Valproate

Valproic acid has been shown to reduce post-injury induced brain damage by reducing inflammation in the brain (Suda et al., 2013), and yet autism is clearly a condition with chronic. How can we resolve that valproic acid can induce autism and reduce brainwide inflammation with measureably improved outcomes? How can it have both neuroprotective and neurotrophic effects?

If we keep in mend that ischemia-reperfusion of the type induced in animal studies leads to massive numbers of damaged brain cells, it would appear that when environmental inducement of microglial activation has no damaged cells as targets, activated cells go after healthy cells. Signals from damaged cells also include a set of cytokines and signalsthat activate microglia (IL-1, TNF-α, GMCSF), whereas most other interleukins deactivate microglia and return them to their ramified state. In the absence of the turn-off signals, the microglial are free to do damage unchecked, and can induce aoptosis in nearby neurons.

This idea is bolstered by the observation that valproaic acid injected in the spines of mice after spinal injury caused improved conservation of hindlimb function (Lu et al., 2013), accompanied by a reduction in the accumulation of microglia/macrophages and astrocytes in the spinal fluid. Similarly, microglial activation after global cerebral schemia was *reduced* by valproaic acid (Xuan et al., 2012).

The mechanism of autism via gestational valproaic acid exposure may have an intermediate step: exposure, microglial apoptosis (Chen et al.,2007), cytokine release, microglial activation, and dendritic extension/neural precursor death. It induces the upregulation of the expression of neurotrophic factors, including glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) from astrocytes (Chen et al., 2006). Valproic acid is thought to be effective in neurodegenerative

diseases such as Alzheimer's, Parkinson's, and HIV dementia by “selectively killing microglia” (Dragunow et al., 2006).

Naturally, autistics who have children should avoid using of valproic acid given the increased combined genetic x exposure risk.

Treatment of Co-Morbid Conditions

Medical doctors attempting to treat co-morbid conditions may find treating some with autism/ASD challenging. At times, a diagnosis of autism/ASD is given to enable a family to access resources they would not qualify for without the diagnosis.

For accuracy in any diagnosis, the symptoms must not be explicable by other diagnosis. This is also a concern for this book, as many of the autism phenotypes may be shared with other conditions due the same underlying genetic pathways. However, DSM-V allows autism diagnosis with, or without specification of “association with” a known medical or genetic condition or environmental factor. This frame of reference neither commits one to think of autism as a subset of other conditions, nor commits one to consider all co-occurring symptoms as being ‘part of’ autism. There are important medical and legal implications for the view one has on such exercises in classification. This is dealt with in the section on “Concepts of Co-Morbidity”, as they are critically important to the definition of clinical populations for clinical studies.

Other specifications include whether the symptoms appear with or without accompanying intellectual impairment, and also with or without accompanying language impairment. These allowances are useful in that they may help refine clinical populations that can be compared for discovery of differentiating molecular etiologies. DSM-V also prescribes levels of severity related to level of care needed.

All of these conditions, including Asperger’s, fall under the umbrella of autism spectrum disorders (ASD) in the DSM-V. This lumping of conditions into ASD can lead to groups of individuals into clinical category full of heterogeneity, making the use of ASD as a well-define comparator group problematic. Each study of autism, and ASD, for example, risks missing more refined important findings. The most reasonable approach for research studies is the focus on association of genes and environmental factors with phenotypes, not heterogeneous populations. The basis of categories and subcategories is not questioned; however, the utility of subsuming these subcategories in “ASD” again begs the question of shared vs. unique molecular and environmental etiologies.

SCN1A Mutations: From Association to Animal Models to Targeted Treatment of Seizures

In a study of Chinese children with SCN1A mutations, Wong et al. (2012) detected a high frequency of ASD (79%) and vaccination-induced encephalopathy (57%) in those DS with SCN1A mutation, and the authors concluded that it would be wise to evaluate further relationships between SCN1A mutations, ASD with epilepsy or vaccination induced encephalopathy. A study of a patient with intractable myoclonic epilepsy and familial migraines revealed a potential role of SCN1A mutation (Frosk et al., 2013).

Note especially the observations by Huynh et al (2008) that

"a large percentage of children who suffered vaccination-induced encephalopathy exhibited clinical progression similar to severe myoclonic epilepsy in infancy (SMEI), which is an epileptic encephalopathy with long seizures accompanied by fever, intractable myoclonic seizure (muscle jerking), and psychomotor decline."

The authors also noted that over 50% of SMEI patients experience their first seizure after DPT vaccination.

In studying this population, the researchers identified mutations in the SCN1A gene in 11 of 14 patients with suspected vaccine encephalopathy. The SCN1A gene encodes a voltage-gated sodium channel protein. It has been hypothesized that SCN1A mutations are predisposing factors "waiting to be triggered by fever or other stresses" (Huynh et al., 2008). A mouse strain with a defective copy of SCN1A (Scn1a+/- mice) was found to exhibit autistic-like behavior, including hyperactivity, stereotyped behaviors, social interaction deficits and impaired context-dependent spatial memory, and aversion to novel food and social odors. The authors also found that a low dose of clonazepam "completely reversed" the phenotypes, indicating that mutational screening in autistic children for SCN1A mutations may aid in alleviating some of the most important types of impairments due to those mutations. The result has been replicated (Han et al., 2012).

This has implications for the treatment of autistic patients with SCN1A mutations: very low-doses may be effective in these cases. A search at clinicaltrials.gov (Jan 2016) found no prospective studies are underway.

Ketogenic Diet, Social Behavior and Seizures

Seizures are example of a condition that is seen as 'co-morbid' but that also appear to shar major etiologic pathways with core ASD symptoms. A high-fat, low carbohydrate ketogenic diet improves social behavior and metabolism in one ASD model mice (Ahn at al., 2014) and reduced seizures in another (Rushkin et al., 2013). Results in humans studies are early, but promising, showing reduced improved learning abilities and social skills in a handful of studies (Napoli et al., 2014).

Behavioral Therapies

Behaviorial therapies are used to help children learn, or relearn language, self-care and social skills, but is typically only moderately successful for severe AD. The focus is primarily on the use of rewards to reinforce desirable behavior.

Lee and Tomblin (2012) showed that the ability for reinforcement learning is retained in ASD, provide a neurobiological basis for such programs. Other studies have shown that autistic retain a great deal more information than many give them credit for. It is easy to underestimate someone on the basis stereotyped behaviors that may not reflect at all on whether a person is paying attention. The sensory input overload, along with the excitatory/inhibitive overdrive, perhaps leading to input signals traveling into the motor cortex as signals for motor movement may be present, but until an autistic is given the

opportunity to demonstrate their knowledge retention, it is best to presume that all types of learning are occurring.

Child Abuse/Neglect

Studies have shown neglect, and stressful, antagonizing treatment by others can lead to microglial activation. How the brain is treated by others influences not only its immediate chemistry. Stress also alters gene expression, inducing activation of microglia. Reduction of microglial activation should reduce long-term damage to dendrites, stripping inhibitory synapses, and devouring neural precursor cells.

Being good to your children can help, and it does not cost anything.

Gene-Informed Treatments

It is now known that autism is medical. The bewildering possible combinations of symptoms, and severity levels, can be clarified greatly via whole genome or whole exome sequencing.

One of the most exciting development for autism/ASD is a long time is the advent of whole genome sequencing (WGS). When clinicians are informed by molecular mechanisms, the knowledge can then be useful toward defining routes to effective therapies and treatments. High resolution, personalized medicine focuses on the link between genotype and phenotypes in the individual patient, not necessarily clinical subtypes (domains)

Genetics lends itself well to fine-resolution diagnostics driven by a desire to provide best treatment for individual phenotypes (i.e., 'medical phenostics').When chromosomal (karyotypic) molecular etiologies of multiple phenotypes are suspected, medical syndromics may be a better term. Neither syndromics or phenostics are mutual exclusive, however, in that individuals with a syndrome may also have a constellation of inherited or de novo mutations that lead to any of the phenotypes associated with conditions within ASD.

When the focus involves the study of genetic or genomic modification, the terms medical genomics seems more appropriate. However, such study groups should be defined by phenotype, and informed by genotype and environmental exposure. The practice of naming syndromes after molecular aberrations in the genome may be convenient, but it may lead to *reductio absurdum*. Each person's unique constellation of inherited and *de novo* risk and modifier mutations – and thus, potentially, each case could reasonably be referred to by that constellation as its own 'syndrome'. This would not be helpful, as the same conditions could be discovered and named in a jumbled bablyosaurus.

This also reveals how some, but not all information is available in genomic assays. In breast cancer, the disease is not defined away by the additional information provided by knowledge of the hormone (estrogen/progesterone) receptor status. Instead, the cancer is referred to, for example, as "ER+" or "ER-", or "triple negative" (ER-, PR-, and HER2Neu-). This knowledge helps oncologists direct the treatment, and I have listed the use of breast cancer receptor status as one of the greatest translational successes in medical history (Lyons-Weiler, 2016).

The same can be envisioned for autism – MECP2+ autism, for example, would inform clinicians of a function-altering mutation in the MECP2 gene. “MECP2-“ could refer to the outright loss (knockout) of that gene for a patient (MECP2 is X-linked). Copy number variations could be referred to as MECP2+’N’, where ‘N’ is the number of copies. The dosage effect of CNV variation is thus communicated as well, without deprecating each and every case of autism to its own developmental or regressive molecular disorder. The combinatorics number of possible mutational /molecular “types” of autism are nearly infinite.

A survey study of parent of autistics and parents of children with no diagnosed disability revealed that parents of autistic children were more likely to use and pay for genetic testing than neurotypical parents. (May, 2012). Parents of autistics also reported being more willing to pay for genetic testing out of pocket if it were a test for aggression of if the test could help develop a tool for early diganosis. Two genes in particular has polymorphisms in the general population that are associated with proactive aggression (monoamine oxidase A gene and CD13). Although IQ is related to use of adaptive behaviors (Kanne et al., 2011), alleles can occur in individuals with and without ASD, and signs of aggression in ASD are independent of most ASD phenotypes with the exception of repetitive behaviors (Kanne et al., 2011).

Although interest is high, parents of autistics in the US are less likely to add a genetic test to their toolbox than those France (Amiet et al., 2014). Some parents may be reluctnant to see research move forward involving genetics and would prefer to have to see research dedicated to environmental factors. However, it makes little sense to environmental risk factors independent of genetic factors, and vice versa. Environmental risks can be aggregated by families just like genetic factors. Studies are needed that examine independent and combined risk of genes and environment through interactions.

The environment is heterogeneous in space and time. Certainly population-level associations may miss some very important environmental factors, however variation across states in ASD could be an important study for specific (narrow) age groups. Lumping variously manifested phenotypes into a broad ‘spectrum’ may be useful for expediting current diagnostic purposes (e.g., family member w/partial autism-like (clinical) or autism-associated (subclinical) features as a risk factor). When genetic researchers study molecular etiologies by focusing on individual phenotypes, and then sets of phenotypes, a much higher resolution picture of disease etiology is seen. Use of ‘unaffected sibs’ can weaken the association if any of the sibs fall in BAP. The focus should be on phenotypes, no necessarily diagnosis; these issue need to be consider in the design of future studies.

Therefore, to eliminate the guesswork, patients could be match to particular combinations of treatments that are likely to be effective given which specific mutations they carry that might be related to their autism symptoms. Enrolled patients could be accrued to a large study that afforded treatment options based on their specific profile – a process called molecular allocation. This would involve whole genome sequencing in case they have other candidate LoF mutations or deletions. This would allow an overall test of all available treatments to molecular subgroups as indicated by their disabled pathways, and also allow for the use of a standardized set of outcome measurements for each phenotype.

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Appendix to Chapter 16

Additional Pharmaceutial and Natural Microglial Suppressors

A very large number of drugs have studies for their potential utility in reducing microglial excitotoxicity. These studies are motived for disease and condition (e.g., brain injury) in which chronic microglial activation is a known culprit. The bulk of the evidence is not from human trials: they are *in vivo* (animal) and *in vitro* (cell culture) studies. This does not constitute medical advice. Although your doctor may be able to prescribe some of these for off-label use, randomized prospective human trials are needed to insure safety, efficacy, and dosage for humans.

Amantadine reduces the activation of microglia, reducing the production of pro-inflammatory compounds and increases the release of Glial Cell Derived Neurotrophic Factor (GDNF; Ossola 2011)

Ossola B. 2011. Amantadine protects dopamine neurons by a dual action: reducing activation of microglia and inducing expression of GDNF in astroglia. Neuropharmacology.61(4):574-82. doi: 10.1016/j.neuropharm.2011.04.030.

Ceftriaxone, an injectable antibiotic, reduced both IL-1β released from microglia and microglia activation during ischemia from brain injury (Lujia et al., 2014).

Lujia Y et al., 2014. Ceftriaxone pretreatment protects rats against cerebral ischemic injury by attenuating microglial activation-induced IL-1β expression. Int J Neurosci. 124(9):657-65. doi: 10.3109/00207454.2013.856009.

Dextromethorphan (a cough suppressant) induces oligodendroglial progenitors and protects them from microglial excitotoxicity (Lisak et al., 2014).

Lisak RP et al., 2014. Effects of dextromethorphan on glial cell function: proliferation, maturation, and protection from cytotoxic molecules. Glia 62(5):751-62. doi: 10.1002/glia.22639.

Ibudilast (a phosphodiesterase inhibitor) suppresses the production of nitric oxide, reactive oxygen species, IL-1β, IL-6, TNF-α and enhanced the production of IL-10, nerve growth factor (NGF), glia-derived neurotrophic factor (GDNF), and neurotrophin (NT)-4 in activated microglia (Mizuno et al., 2004).

Mizuno T et al., 2004. Neuroprotective role of phosphodiesterase inhibitor ibudilast on neuronal cell death induced by activated microglia. Neuropharmacology. 46(3):404-11.

Lithium significantly inhibits lipopolysaccharide (LPS)-induced microglial activation by by activating the PI3K/Akt/FoxO1 pathway and suppressing LPS-induced TLR-4 expression (Dong et al., 2014)

Dong H et al., 2014. Lithium ameliorates lipopolysaccharide-induced microglial activation via inhibition of toll-like receptor 4 expression by activating the PI3K/Akt/FoxO1 pathway. J Neuroinflammation. 2014 Aug 14;11:140. doi: 10.1186/s12974-014-0140-4.

A number of studies have shown that altrexone (LDN) has an antagonist effect on the TLR-4 receptor on microglia, inhibiting microglial activation at low doses (e.g., Mattioli et al., 2010).

Mattioli TA et al., 2010. Ultra-low dose naltrexone attenuates chronic morphine-induced gliosis in rats. Mol Pain. 2010 Apr 16;6:22. doi: 10.1186/1744-8069-6-22.

Magnesium sulfate inhibited the release of iNOS, nitric oxide, prostaglandin E2, interleukin 1β, and tumor necrosis factor α, and the expression of inducible nitric oxide synthase mRNA in LPS-activated microglia. It also inhibited the translocation of NF-κB from the cytoplasm to the nucleus in a dose-dependent manner.

Gao F, 2013. Magnesium sulfate provides neuroprotection in lipopolysaccharide-activated primary microglia by inhibiting NF-κB pathway. J Surg Res. 184(2):944-50. doi: 10.1016/j.jss.2013.03.034.

Minocycline (an antibiotic) inhibited microglial activation by 47% after 6-hydroxydopamine injection into mouse striatum. A small, open-label clinical trial showed now no improvement in patients with autism (Pardo et al., 2013); however, the researchers also included vitamin B6 to ameliorate side effects, which confounds the design. The results did include a significant – and consistent - difference in brain-derived neurotrophic factor in cerebral spinal fluid.

He Y et al., 2001. Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. Brain Res 909(1-2):187-93.

Pardo CA, et al., 2013. A pilot open-label trial of minocycline in patients with autism and regressive features. J Neurodev Disord. 5(1):9. doi: 10.1186/1866-1955-5-9.

Oxymatrine inhibits production of nitric oxide, iNOS, PGE2, COX-2, TNF-α, IL-1β and IL-6 in LPS-stimulated BV2 microglial cells (Fan et al., 2009) and down-regulates TLR-2 and TLR-4 TLR4, TLR2, MyD88, and NF-kappaB and protects rat brains against focal ischemia from internal injury.

Fan H et al.,2009. Oxymatrine downregulates TLR4, TLR2, MyD88, and NF-kappaB and protects rat brains against focal ischemia. Mediators Inflamm. 2009:704706. doi: 10.1155/2009/704706.

Dong XQ et al., 2013. Anti-inflammatory Effects of Oxymatrine Through Inhibition of Nuclear Factor-kappa B and Mitogen-activated Protein Kinase Activation in Lipopolysaccharide-induced BV2 Microglia Cells. Iran J Pharm Res. 12(1):165-74.

Risperidone (antipsychotic drug) significantly inhibits interferon gamma-induced microglial activation in vitro

Kato T et al., 2007. Risperidone significantly inhibits interferon-gamma-induced microglial activation in vitro. Schizophr Res92(1-3):108-15.

Pioglitazone (Actos) inhibits nitric oxide, iNOS, TNF-α, IL-6 and IL-1β production in LPS-stimulated microglia by blocking p38 mitogen-activated protein kinase signaling pathways

Ji H et al., 2010. PPARγ agonist pioglitazone inhibits microglia inflammation by blocking p38 mitogen-activated protein kinase signaling pathways. Inflamm Res. 59(11):921-9. doi: 10.1007/s00011-010-0203-7.

In a screen of 1,040 compounds for effectiveness and low-toxicity to microglia, Samanani et al.,found that 123 compounds reduced TNF-α levels of LPS-activated microglia by over 50%; 69 of these were cytotoxic to microglia. The remaining 54 were assessed to be non-toxic. Pironolactone was found to inhibit TNF-α release (by 50% to 60% at 10 μM) from LPS-activated microglia

Samanani S et al., 2013. Screening for inhibitors of microglia to reduce neuroinflammation. CNS Neurol Disord Drug Targets. 12(6):741-9.

Propentofylline inhibits release of IL-1β and TNF-α from microglia activated by LPS (Si et al., 1998)

Si Q et al., 1998. Differential regulation of microglial activation by propentofylline via cAMP signaling. Brain Res. 812(1-2):97-104.

Rifampicin, an antibiotic inhibited nitric oxide, iNOS, COX-2, IL-1β, TNF-α and prostaglandin E2 from LPS- stimulated microglia

Bi W et al., 2011. Rifampicin inhibits microglial inflammation and improves neuron survival against inflammation. Brain Res. 1395:12-20. doi: 10.1016/j.brainres.2011.04.019.

The cholesterol-lowering drug Simvastatin was found by Li et al. (2009) to reduce IL-1β and to inhibit the activation of microglial cells in traumatic brain injury

Li B et al., 2009. Simvastatin attenuates microglial cells and astrocyte activation and decreases interleukin-1beta level after traumatic brain injury. Neurosurgery65(1):179-85; discussion 185-6. doi: 10.1227/01.NEU.0000346272.76537.DC.

A class of antidepressants (SSRI) potently inhibit microglial TNF-α and nitric oxide production in microglia

Tynan RJ A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. Brain Behav Immun. 26(3):469-79. doi: 10.1016/j.bbi.2011.12.011.

Tetracyclines (a class of antibiotic) inhibit ischemic stroke-induced activation of microglia

Yrjänheikki J et al., 1998. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. Proc Natl Acad Sci U S A. 95(26):15769-74.

Valcyte (valganciclovir) and Cytovene (ganciclovir) inhibit microglial activation in a mouse model of autoimmune encephalitis.

Ding Z et al., 2014. Antiviral drug ganciclovir is a potent inhibitor of microglial proliferation and neuroinflammation. J Exp Med;211(2):189-98. doi: 10.1084/jem.20120696.

Natural Compounds

What we eat and drink represents the single largest environmental exposure over the course of our lives. In this section, I review the scientific studies on the effects of food, supplements, and other non-medical environmental factors that reduce microglial activation. As for the review of available drugs, this does not constitute medical advice; consult with your doctor before pursuing any significant change in the care of your child or yourself.

Many of the pharmaceuticals above and the food and supplement sources of inhibition of microglial activation work via different pathways. Those that medicate TLR-2 and TLR-4 expression, for example, may be complementary to drugsor supplements that help reverse mitochondrial dysfunction or directly influence glutamate pathways (e.g., pomaglumetad).

Acupuncture reduced MAC-1, a measure of microglial activation in mice in which activation was induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Kang JM et al., 2007. Acupuncture inhibits microglial activation and inflammatory events in the MPTP-induced mouse model. Brain Res. 1131(1):211-9.

Andrographolide, and extract from the herb *Andrographis paniculata*, inhibits microglial activation (Wang et al., 2004).

Wang T et al., 2004. Andrographolide reduces inflammation-mediated dopaminergic neurodegeneration in mesencephalic neuron-glia cultures by inhibiting microglial activation. J Pharmacol Exp Ther. 308(3):975-83.

Acetate reduced the pro-inflammatory cytokine IL-1β by and inhibited microglial activation in a rat model of Lyme disease (Brissette et al.,2012). Acetate can be distilled from vinegar.

Brissette CA et al., 2012. Acetate supplementation reduces microglia activation and brain interleukin-1β levels in a rat model of Lyme neuroborreliosis. J Neuroinflammation. 7;9:249. doi: 10.1186/1742-2094-9-249.

Ar-turmerone (found within turmeric) not only reduces microglial activation by blocking of NF-κB, JNK, and p38 MAPK (Park et al., 2012; but also stimulates the proliferation of neural stem cells (Hucklenbroich et al., 2014).

Hucklenbroich J et al., 2014. Aromatic-turmerone induces neural stem cell proliferation in vitro and in vivo. Stem Cell Res Ther. 5(4):100. doi: 10.1186/scrt500.

Park SY et al., 2012. Aromatic-turmerone's anti-inflammatory effects in microglial cells are mediated by protein kinase A and heme oxygenase-1 signaling. Neurochem Int. 2012 Oct;61(5):767-77. doi: 10.1016/j.neuint.2012.06.020.

Park SY et al., 2012. Anti-inflammatory effects of aromatic-turmerone through blocking of NF-κB, JNK, and p38 MAPK signaling pathways in amyloid β-stimulated microglia. Int Immunopharmacol. 14(1):13-20. doi: 10.1016/j.intimp.2012.06.003.

Baicalein (flavonoid from skullcap herb) almost complete blocks LPS-induced activation of microglia (Li et al. (2005).

Li FQ et al., 2005. Inhibition of microglial activation by the herbal flavonoid baicalein attenuates inflammation-mediated degeneration of dopaminergic neurons. J Neural Transm (Vienna). 112(3):331-47.

Beta-glucans, found in edible mushrooms, reduces toll-like receptor-2 (TLR-2- and TLR-4) , and shuts down pro-inflammatory cytokine production by microglia (Shah et al., 2009).

Shah VB et al., 2009. beta-Glucan attenuates TLR2- and TLR4-mediated cytokine production by microglia Neurosci Lett. 458(3):111-5. doi: 10.1016/j.neulet.2009.04.039.

A 2% diet of blueberries significantly reduced microglial activation in the hippocampus (Willis et al., 2010). It had previously been observed to reduce microglial activation and enhance dopamine recovery in combination with spirulina by Strömberg et al. (2005).

Strömberg I, et al., 2005. Blueberry- and spirulina-enriched diets enhance striatal dopamine recovery and induce a rapid, transient microglia activation after injury of the rat nigrostriatal dopamine system. Exp Neurol. 196(2):298-307.

Willis LM et al., 2010. Blueberry supplementation attenuates microglial activation in hippocampal intraocular grafts to aged hosts. Glia. 58(6):679-90. doi: 10.1002/glia.20954.

Cannabinoids (non-psychoactive component of *Cannabis*). All of the cannabinoids studied decreased LPS-induced nitrite generation. The effects were extreme enough to prevent a spatial learning task. THC, the psychoactive component of Cannabis, not included in the study, induces microglial activation (Cutando et al., 2013).

Martín-Moreno AM et al.,2011. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. Mol Pharmacol. 79(6):964-73. doi: 10.1124/mol.111.071290.

Cutando L 2013. Microglial activation underlies cerebellar deficits produced by repeated cannabis exposure. J Clin Invest. 123(7):2816-31. doi: 10.1172/JCI67569.

Chronic caffeine ingestion reduces microglial density throughout the brain, and returned microglial the ramified state with (shortening process length and decreasing branching; Steger et al., 2014).

Steger R et al.,2014. Chronic caffeine ingestion causes microglia activation, but not proliferation in the healthy brain. Brain Res Bull. 106:39-46. doi: 10.1016/j.brainresbull.2014.05.004.

Curcumin (found within turmeric) blocks the production of nitric oxide, TNF-α, IL-1α and IL-6 in IFN-gamma- and reduced LPS-stimulated microglial activation, but did not reduce dopamine-directed neuronal cell death and sodium nitroprosside (SNP)-induced NO generation (Lee et al., 2007).

Lee HS et al., 2007. Neuroprotective effect of curcumin is mainly mediated by blockade of microglial cell activation. Pharmazie. 62(12):937-42.

Gastrodia elata **(a Chinese herb, aka: Tian Ma) suppresses of nNOS and microglia activation in rats treated with kainic acid (Hseih et al., 2005))**

Hsieh CL et al., 2005. Gastrodia elata BL mediates the suppression of nNOS and microglia activation to protect against neuronal damage in kainic acid-treated rats. Am J Chin Med. 33(4):599-611.

Fisetin, a flavonoid decreased nitric oxide, iNOS, TNF-α, IL-1β, COX-2, and prostaglandin E2 in LPS-stimulated microglia (Zheng et al., 2008). It is found flavonol found in low levels in some fruits and vegetables such as apples, blueberries, cumcumber, grapes, kiwi, mangoes, onions, persimmon and strawberries. It also reduced aluminum neurotoxicity (Prakash et al., 2013).

Prakash D et al., 2013. Fisetin enhances behavioral performances and attenuates reactive gliosis and inflammation during aluminum chloride-induced neurotoxicity. Neuromolecular Med. 15(1):192-208. doi: 10.1007/s12017-012-8210-1.

Zheng et al., 2008. Suppressive effects of flavonoid fisetin on lipopolysaccharide-induced microglial activation and neurotoxicity. Int Immunopharmacol. 8(3):484-94. doi: 10.1016/j.intimp.2007.12.012.

Genistein, a flavonoid from soy, reduces dopaminergic neuronal damage from microglial activation (Wang X et al., 2005).

Wang X et al., 2005. Genistein protects dopaminergic neurons by inhibiting microglial activation. Neuroreport. 2005 Feb 28;16(3):267-70.

Ginsenoside Rg3 (from *Panax ginseng*)

Green tea contains a compound called Epigallocatechin gallate inhibits LPS induced microglial activation, and reduced dopaminergic neuronal injury (Li et al., 2004). However, green tea contains fluoride, which can induce microglial activation.

Li R et al., 2004. (-)-Epigallocatechin gallate inhibits lipopolysaccharide-induced microglial activation and protects against inflammation-mediated dopaminergic neuronal injury. J Neurosci Res. 78:723–31.

High-molecular weight hyaluronan reduces LPS-induced microglial activation by a TLR-4 receptor mechanism

Austin JW et al., 2012. High molecular weight hyaluronan reduces lipopolysaccharide mediated microglial activation. J Neurochem. 2012 Jul;122(2):344-55. doi: 10.1111/j.1471-4159.2012.07789.x.

Icariin (extract of Epimedium, horny goat weed inhibits nitric oxide, prostaglandin E2, reactive oxygen species, IL-1β, IL-6 and TNF-α in LPS-activated microglia. Icariin also reduced the degeneration of cortical neurons induced by LPS-activated microglia in neuron-microglia co-culture system (Zeng et al., 2010).

Zeng KW et al., 2010. Icariin attenuates lipopolysaccharide-induced microglial activation and resultant death of neurons by inhibiting TAK1/IKK/NF-kappaB and JNK/p38 MAPK pathways. Int Immunopharmacol. 10(6):668-78. doi: 10.1016/j.intimp.2010.03.010.

Inflexin, a putative antiinflammatory agent isolated from *Isodon excisus*, significantly inhibits the release of nitric oxide from microglia, suppressing neuroinflammation via inhibition of NF-kappaB activation and Akt pathway (Ko et al., 2010).

Ko HM et al., 2010. Inflexin attenuates proinflammatory responses and nuclear factor-kappaB activation in LPS-treated microglia. Eur J Pharmacol. 633(1-3):98-106. doi: 10.1016/j.ejphar.2010.02.011.

Isodojaponin D (from the herb *Isodon japonicus*) reduced LPS-induced IL-1beta, IL-6, and TNF-alpha proinflammatory citations, and also reduced MAPK activation in a preventive manner (Lim JY et al., 2010).

Lim JY, et al.,2010. The new diterpene isodojaponin D inhibited LPS-induced microglial activation through NF-kappaB and MAPK signaling pathways. Eur J Pharmacol. 642(1-3):10-8. doi: 10.1016/j.ejphar.2010.05.047.

Lycopene reduced microglial activity and reduced nitrite production 61% in microglia after injury-induced ischemia (Hsiao et al., 2004).

Hsiao G et al., 2004. A potent antioxidant, lycopene, affords neuroprotection against microglia activation and focal cerebral ischemia in rats. In Vivo.18(3):351-6.

Luteolin (flavonoid supplement in Chamomile teas) inhibits LPS-induced release of nitric oxide, COX-2, TNF-α, IL-1β, IL-6 and prostaglandin E2 from microglia

Jang S et al., 2008. Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. Proc Natl Acad Sci U S A. 2008 May 27;105(21):7534-9. doi: 10.1073/pnas.0802865105.

Zhu LH et al., 2011. Luteolin inhibits microglial inflammation and improves neuron survival against inflammation. Int J Neurosci. 121(6):329-36. doi: 10.3109/00207454.2011.569040.

N-acetyl-glucosamine inhibits LPS microglial activation.

Yi HA et al., 2005. Inhibitory effects of glucosamine on lipopolysaccharide-induced activation in microglial cells. Clin Exp Pharmacol Physiol. 32(12):1097-103.

Obovatol (from *Magnolia officinalis*) attenuates microglia-mediated LPS-induced cytokine production, and neuroinflammation, impacting a broad number of additional effects (Ock et al., 2010).

Ock J et al., 2010. Obovatol attenuates microglia-mediated neuroinflammation by modulating redox regulation. Br J Pharmacol. 159(8):1646-62.

Ock J et al., 2010. Obovatol attenuates microglia-mediated neuroinflammation by modulating redox regulation. Br J Pharmacol. 2010 Apr;159(8):1646-62.Palmitoylethanolamide (PEA) reduces microglial activation induced by formalin or MPTP

Reishi (*Ganoderma lucidum*) inhibits nitric oxide, IL-1β and TNF-α release from microglia activated with LPS (Zhang et al., 2011).

Zhang R et al., 2011. Ganoderma lucidum protects dopaminergic neuron degeneration through inhibition of microglial activation. Evid Based Complement Alternat Med. 2011:156810. doi: 10.1093/ecam/nep075.

Resveratrol reduced two types of microglial activation (Capiralla et al., 2012)

Capiralla H et al., 2012. Resveratrol mitigates lipopolysaccharide- and Aβ-mediated microglial inflammation by inhibiting the TLR4/NF-κB/STAT signaling cascade. J Neurochem. 120(3):461-72. doi: 10.1111/j.1471-4159.2011.07594.x.

Sesamin, from sesame seed oil, kept microglial cells in the ramified state under activation conditions (intracerebral hemorrhage).

Ohnishi M et al., 2013. Neuroscience. 2013 Mar 1;232:45-52. doi: 10.1016/j.neuroscience.2012.11.057.

Silymarin (from milk thistle herb) inhibits LPS-induced activation of microglia.

Wang MJ et al., 2002. Silymarin protects dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting microglia activation. Eur J Neurosci. 16(11):2103-12.

Vinpocetine (a supplment) inhibited the production of nitrite oxide and inflammatory cytokines IL-1β, IL-6 and TNF-α in BV-2 microglia (Zhao et al., 2011).

Zhao YY et al., 2011. TSPO-specific ligand vinpocetine exerts a neuroprotective effect by suppressing microglial inflammation. Neuron Glia Biol. 7(2-4):187-97. doi: 10.1017/S1740925X12000129.

Vitamin E

Li Y et al., 2001. Vitamin E suppression of microglial activation is neuroprotective. J Neurosci Res. 2001 Oct 15;66(2):163-70.

Wogonin, a flavonoid from skullcap herb.

Lee H et al., 2003. Flavonoid wogonin from medicinal herb is neuroprotective by inhibiting inflammatory activation of microglia. FASEB J. 17(13):1943-4.