# Lecture 4b - Mitochondrial involvement in autism spectrum disorders (ASDs)

Rachel Nicoll PhD

# Dramatically increased prevalence: described as an 'autism tsunami'

- According to the US Centres for Disease Control, ASD is the **fastest growing developmental disability, affecting 1 in 59 children (1970s: 1 in 5000)**, with males affected 4.5 times more often than females. Prevalence has increased 10-17% each year over the last several years. (Griffiths KK, Oxid Med Cell Longev, 2017; Hollis F, Curr Opin Neurobiol, 2017; Rose S, Mol Diag Ther, 2018; Blaxill M, J Autism Dev Disord, 2021)
- The various levels of autism, including Asperger's syndrome, are combined under the name of autism spectrum disorders (ASDs).
- Autism is a pervasive lifelong developmental disorder characterised by difficulties with social interaction and communication and by restricted and repetitive behaviour. It is associated with a combination of genetic, immune and environmental factors (infections, autoimmune disease, immunoglobulin deficiency, environmental toxins) which affect cell signalling, metabolic, immune and epigenetic triggers. (Chaste P, Dialog Clin Neurosci, 2012; Rose S, Mol Diag Ther, 2018; Griffiths KK, Oxid Med Cell Longev, 2017)
- In the brain, ASD is characterised by altered development, structure and/or function of synapses, resulting in impaired synaptic and dendritic organisation (Hollis F, Curr Opin Neurobiol, 2017).

# Conventional medicine: the genomic approach to autism

- In the US, >$1 billion has been spent on genetic research into autism over the past 10 years. This has shown that **hundreds of genes may play a role,** with the collective contribution of all genes in ASD being around 40%. **But no single gene accounts for more than 1–2% of autism**.
- **Our DNA cannot change this fast. Therefore, the rise in the prevalence of autism spectrum disorders (ASDs) is not genetic.** Recent twin studies show that environmental factors are responsible for about 60% of ASDs.
- These genes found to predispose children to autism are known as **'autism susceptibility genes**'. So far scientists have identified 102 of these genes. But studies have failed to confirm a high rate of genetic mutations in ASDs or a single gene or chromosomal defect. Where there are mutations, they are rarely the cause of the condition.
- There is officially no cure for autism because the underlying cause is 'unknown'. Medical therapy is currently limited to targeting behavioural symptoms. Yet between 3-25% of children reportedly lose their ASD diagnosis and enter the normal range of cognitive, adaptive and social skills. The work of Robert Naviaux shows that these children can be helped.
- Although many urinary and blood biomarkers may be dysregulated in ASDs, none has been found to predict accurately or detect the condition, although many studies have found that individuals with ASD display impaired methylation or detoxification, increased oxidative stress or abnormalities in redox regulation.

(Rose S, Mol Diag Ther, 2018; Hollis F, Curr Opin Neurobiol, 2017; Griffiths KK, Oxid Med Cell Longev, 2017; Helt M, Neuropsychol Rev, 2008)

# Alternative hypotheses

- Gut involvement
- Dysregulated serotonin
- Environmental toxins
- Immune or autoimmune involvement: dysregulated IL-17A

# Autism and gut involvement

- Gastrointestinal complications are one of the major ASD comorbidities; their prevalence ranges from 9% to 70% and correlates with behaviours consistent with the autistic endophenotype. Strong gut-brain cross-talk results, which is responsible for excessive production of short-chain fatty acids such as propanoic acid by abnormal gut flora, which worsens behavioural, neurochemical and mitochondrial dysfunction and generate free radicals cause immune system dysfunction leading to synthesis of various pro-inflammatory cytokines and chemokines. This in turn causes activation of microglia. (Bhandari R, Adv Neurobiol, 2020)
- Gut symptoms are common in ASDs and ETC Complex I protein quantities are found to be increased in intestinal tissue (Rose S, Mol Diag Ther, 2018).
- *Clostridia* spp tend to be over-represented in the ASD GI tract, while there are high levels of propionic acid, a short-chain fatty acid produced by gut bacteria. Propionic acid induces ASD-like behaviour in rodents, with similar abnormalities in redox, metabolic, immune and neurophysiological systems as are seen in ASD children. (Rose S, Mol Diag Ther, 2018)
- Butyrate, another short-chain fatty acid produced by gut bacteria, could increase expression of genes involved with mitochondrial fission, mitophagy, oidative stress and energy metabolism in ASDs. (Rose S, Mol Diag Ther, 2018)

# Autism and serotonin dysregulation

- Many studies have demonstrated that children with autism have increased serum serotonin levels; many people with autism have a variant in their gut serotonin transporter which boosts the amount of serotonin in the blood.
- In the foetus, serotonin helps neurons form and travel to their correct locations; it also helps them link to other neurons at synapses. Both too much and too little serotonin can be harmful.
- Low serotonin levels is often seen in the brains of people with autism who show repetitive behaviours and social difficulties. Brain-imaging studies indicate that some autistic children make too little serotonin in the brain, and in others, too little serotonin binds to its brain receptors.
- When autistic adults adopt a diet low in tryptophan (precursor of serotonin), their repetitive behaviours worsen and their irritability increases. So possibly a high serotonin diet increases blood serotonin levels but not brain levels.
- Some research suggests that the reason for the paradoxical levels of serotonin in individuals with autism (high in the blood, low in the brain) is because of low vitamin D. Vitamin D activates the transcription of the serotonin-synthesising gene tryptophan hydroxylase 2 (TPH2) in the brain and represses the transcription of TPH1 in tissues on the other side of the blood-brain barrier, i.e. in the blood and other tissues).

(Guo M, Brain Res Bull, 2018; Patrick RP, FASEB J, 2014)

# Autism and environmental toxins

- A high ASD prevalence has been reported from countries with water fluoridation as well as from endemic fluorosis areas.
- Aluminium content is high in ASD brains. Use of paediatric vaccines with an aluminium adjuvant correlates to increased ASD incidence.
- Both fluoride and aluminium interfere with a number of glycolytic enzymes, resulting in a significant suppression of cellular energy production. The synergistic interactions of fluoride and aluminium increase the potential neurotoxic effect, particularly in children. Aluminofluoride complexes have effects on cell signalling, neurodevelopment and neuronal function.
- Risk of ASDs was associated with prenatal exposure to glyphosate and various pesticides.
- Increased human exposure to electromagnetic fields has been implicated in the increasing incidence of ASDs. Pre- and neonatal exposure caused a lack of normal sociability and decreased exploratory activity in mice.

(Strunecka A, J Appl Biomed. 2016; Mold M, J Trace Elem, 2018; Gherardi AK, Front Neurol, 2015; Shaw CA, Immunol Res, 2013; Seneff S, Agricult Sci, 2015; von Ehrenstein OS, BMJ, 2019; Herbert MR, Pathophysiol, 2013; Alsaeed I, Int J Dev Neurosci. 2014)

# Autism and immune or autoimmune involvement

- **Studies have shown that in the brain and CSF of autistic patients** there is evidence of both **inflammation and immune dysregulation**, with decreased circulating T cells, natural killer cells and T helper cells, and abnormal accumulation of T lymphocytes in other tissues.
- **Mothers of children with ASDs are significantly more likely to have an autoimmune disease** than women of children without ASDs.
- **Another study found that pregnant women who had an infection, an inflammatory response or an activated immune system through vaccination, had a higher risk of having an autistic child. Maternal cytokines can cross the placenta and enter the immature foetal brain.**
- **Pregnant women** may also have circulating **brain-reactive autoantibodies**. Although these antibodies can never access brain tissue in the mother, they **can access the foetal brain during pregnancy**. Animal models have shown that *in utero* exposure to maternal brain-reactive **antibodies can permanently alter brain anatomy and cause persistent behavioural or cognitive dysfunction**.
- Immunotherapy improved some clinical symptoms of autism and completely normalised others.

(Hughes HK, Front Cell Neurosci, 2018; Enstrom AM, Curr Opin Investig Drugs. 2009; Gata-Garcia A, Front Immunol, 2019; Braunschweig D, Arch Neurol, 2012; Ashwood P, Autoimmun Rev, 2004; Mutlu ZS, J Exp Basic Med Sci, 2021; Meltzer A, Neuropsychopharmacology, 2017; Antonucci N, Madridge J Vaccines, 2019)

# Interleukin 17A (IL-17A) in children with ASDs

- **The key cytokine in maternal autoimmune disease seems to be IL-17A, which can also enter the foetal brain.** We saw that maternal infection or autoimmune disease could induce autism in their child.
- **Around 50% of children with ASDs were found to have higher levels of IL-17A than healthy children and levels correlated with autism severity.**
- Injection of recombinant IL-17A into foetal brain ventricles in animals resulted in ASD-like behavioural and histopathological abnormalities.
- The task of IL-17A signalling is to play a protective role in adaptive immunity. However if this is dysregulated, an over-production of IL-17A could trigger autoimmune disease and inflammatory disorders and in pregnant women may be responsible for foetal ASD development, since IL-17A can cross the placental barrier.

(Mutlu ZS, J Exp Basic Med Sci, 2021; Al-Ayadhi LY, J Neuroinflammation, 2012; Sasaki T, Mol Brain, 2020)

# Autism and serotonin (1)

- Many studies have demonstrated that children with autism have increased serum serotonin levels. Blood serotonin levels are controlled in part by the serotonin transporter, which moves serotonin from the gut, where most serotonin is made, into blood cells; many people with autism have a variant in their gut serotonin transporter which boosts the amount of serotonin in the blood. (Guo M, Brain Res Bull, 2018; Patrick RP, FASEB J, 2014).
- In the foetus, serotonin helps neurons form and travel to their correct locations; it also helps them link to other neurons at synapses. Both too much and too little serotonin can be harmful; mice exposed to excess serotonin *in utero* show altered development in a brain region that responds to whisker movements.
- Yet some studies point to low serotonin levels in the brains of people with autism who show repetitive behaviours and social difficulties. Brain-imaging studies indicate that some autistic children make too little serotonin in the brain, and in others, too little serotonin binds to its brain receptors.
- When autistic adults adopt a diet low in tryptophan (precursor of serotonin), their repetitive behaviours worsen and their irritability increases. They also show altered patterns of brain activity in regions involved in face processing. So possibly a high serotonin diet increases blood serotonin levels but not brain levels.
- Furthermore, the use of SSRI antidepressants, particularly Prozac, to increase serotonin in the brain was ineffective in clinical trials.
- What is going on?

# Autism and serotonin (2)

- Some research suggests that the reason for the paradoxical levels of serotonin in individuals with autism (high in the blood, low in the brain) is because of low vitamin D.
- Vitamin D activates the transcription of the serotonin-synthesising gene tryptophan hydroxylase 2 (TPH2) in the brain and represses the transcription of TPH1 in tissues on the other side of the blood-brain barrier, i.e. in the blood and other tissues (Patrick RP, FASEB J, 2014).
- The involvement of vitamin D in serotonin levels may also help to explain some of the gender differences in autism. Oestrogen can also increase the expression of TPH2, which helps to synthesise serotonin; this can protect the female brain from serotonin deficiency, accounting for the heightened incidence of autism in boys.

# Mitochondrial involvement in ASDs

- **Up to 80% of children with ASDs demonstrate evidence of mitochondrial dysfunction**. In general, individuals with autism have an inability to produce sufficient cellular energy, as less oxygen is consumed, there is impaired fatty acid oxidation and higher levels of ROS (as hydrogen peroxide) are produced.
- The vast **majority of studies show decreased ETC activity but a few show increased activity. None show normal activity.**
- **MRI studies have confirmed brain mitochondrial dysfunction in ASDs, and particularly abnormal energy production, with elevated lactate**. Elevated plasma lactate is sometimes used as a biomarker in children with ASDs. Abnormalities in fatty acid metabolism have also been noted, which may account for the common deficiency in carnitine found in ASD patients.
- There is also evidence of **abnormal intracellular calcium signalling** in autism and pathologically increased cytochrome c oxidase activity and oxidative stress.
- Huge numbers of mitochondria are required in the developing brain for the rapid construction of axons and dendrites for the nervous system. They accumulate around synapses in response to increased calcium levels during synaptic activity.

(Hollis F, Curr Opin Neurobiol, 2017; Rose S, Mol Diag Ther, 2018; Griffiths KK, Oxid Med Cell Longev, 2017; Barone R, Int J Mol Sci, 2019; Delhey L, J Clin Med, 2017; Yui K, Mini Rev Med Chem, 2015; Zhang B, J Neuroinflammation, 2010; Griffiths KK, Oxid Med Cell Longev, 2017)

# ASDs and brain mitochondria

- MRI studies have shown brain mitochondrial dysfunction in ASDs, and particularly abnormal energy production, with elevated lactate. There may be decreased aconitase and pyruvate dehydrogenase. There is also increased oxidative damage in brain tissue, which is associated with mitochondrial dysfunction, and a decrease in SOD and ETC activity. (Rose S, Mol Diag Ther, 2018)
- In ASD brains, mitochondrial dysfunction tends to be region-specific. In the frontal cortex, ETC Complexes I, III and V can be reduced but predominantly Complex I, while in the cerebellum reduction in Complexes III and V were more prevalent, but in the temporal lobe all Complexes can exhibit reduced activity. Decreased protein expression of Complexes I, III, IV and V were also found in the motor cortex, thalamus and cingulate gyrus. Some of these reductions in ETC Complex function and pyruvate dehydrogenase could be as much as 30% in the frontal cortex. (Hollis F, Curr Opin Neurobiol, 2017; Griffiths KK, Oxid Med Cell Longev, 2017)
- Both increased markers of oxidative stress, enhanced oxidative DNA damage and impaired antioxidant mechanisms, particularly mtSOD, have been seen in several brain areas but particularly in the cerebellum and temporal cortex. (Hollis F, Curr Opin Neurobiol, 2017; Griffiths KK, Oxid Med Cell Longev, 2017)
- There could also be decreased protein expression of genes responsible for mitochondrial homeostasis with increased mitochondrial gene copy number, possibly attempting to compensate for the reduced activity. (Griffiths KK, Oxid Med Cell Longev, 2017)

# ASDs and non-brain mitochondria

- Skeletal muscle mitochondria showed potential defects in all 5 ETC Complexes in autistic children, with Complex I deficiency being the most common. Leukocyte, lymphocyte or buccal mucosa mitochondria can also show impaired activity of Complexes I, III and IV. (Griffiths KK, Oxid Med Cell Longev, 2017)
- Children with ASDs demonstrated significantly greater variation in mitochondrial activity compared to controls, with more than expected ASD children having enzyme activity outside of the normal range for the TCA cycle enzyme citrate synthase (24%), Complex I (39%) and Complex IV (11%). Poorer adaptive skills were associated with both higher and lower Complex IV activity and lower Complex I activity. Poorer social function and behavior was associated with relatively higher citrate synthase activity. Both mitochondrial underactivity and overactivity in seen ASD, which can manifest in a number of body tissues, pointing up that autism appears to be a systemic condition. There is also an association with ETC Complex deficiencies of proteins encoded by mtDNA. Dysregulation of mTOR signalling has also been found.
- A US team also found that the regulatory peptide neurotensin, secreted in the gut and hypothalamus, is increased in autistic children and also induces release of extracellular mtDNA that could act as an autoimmune trigger. Serum from young autistic patients contained both mtDNA and anti-mitochondrial antibodies, as compared to normal controls.
- Elevated plasma lactate is sometimes used as a biomarker in children with ASDs, and could indicate upregulated carbohydrate metabolism or an OXPHOS defect. There may also be a high lactate/pyruvate ratio, alanine and ammonia and several elevated urinary organic acids indicative of mitochondrial dysfunction. Abnormalities in fatty acid metabolism have also been noted, which may account for the common deficiency in carnitine found in ASD patients. (Rose S, Mol Diag Ther, 2018; Griffiths KK, Oxid Med Cell Longev, 2017)
- The vast majority of studies show decreased ETC activity but a few show increased activity. None show normal activity. (Griffiths KK, Oxid Med Cell Longev, 2017)

# ASDs and intracellular calcium

- Calcium defects could result in the decreased neurotransmitter signalling seen in ASDs, especially in neurons that have a high rate of firing; this could explain the relative increase in excitatory-to-inhibitory neuron ratio seen in patients with ASDs.
- Autism has also been associated with abnormalities in the expression of several genes involved with calcium signalling or homeostasis. The calcium-dependent mitochondrial aspartate/glutamate carrier (AGC1) links defects in calcium regulation with mitochondrial dysfunction.
- There is also an association between abnormal calcium signalling in autism and pathologically increased cytochrome c oxidase activity and oxidative stress.

(Griffiths KK, Oxid Med Cell Longev, 2017)

# ASDs and oxidative stress

- Oxidative stress induces mitochondrial dysfunction, while dysfunctional mitochondria produce oxidative stress in a PFL (Rose S, Mol Diag Ther, 2018).
- Children with ASDs regularly have low GSH, while the oxidised form of glutathione is elevated. Post mortem brains samples also show GSH abnormalities, with oxidative damage to mtDNA. (Rose S, Mol Diag Ther, 2018)

# Correspondence between ASDs and mitochondrial diseases

- The prevalence of mitochondrial diseases in the population of ASD sufferers was 5.0%, much higher than found in the general population (c0.01%). The prevalence of developmental regression (52%), seizures (41%), motor delay (51%), gastrointestinal abnormalities (74%), female gender (39%) and elevated lactate (78%) and pyruvate (45%) was significantly higher in these children, compared with the general ASD population; a contributory factor in MELAS, the most common mitochondrial disease, is lactic acidosis. They also demonstrate a different redox metabolism profile. (Rossignol DA, Mol Psychiatr, 2012; Rose S, Mol Diag Ther, 2018)
- Most of these cases (79%) were not associated with genetic abnormalities, although a few had defects in mtDNA (Rossignol DA, Mol Psychiatr, 2012; Griffiths KK, Oxid Med Cell Longev, 2017).
- The combination of fever and mitochondrial disease can increase the risk of developing ASDs; in a small study, 71% of autistic children with mitochondrial disease regressed after fever. 33% regressed with vaccination if it also generated a fever but none showed regression with vaccination unless a febrile response was present. (Shoffner J, J Child Neurol, 2010; Griffiths KK, Oxid Med Cell Longev, 2017)

# Fever and autism spectrum disorders (ASDs)

- **Fever in autistic children can generate improvements** in cognition, communication, social interaction and behaviour, possibly through increased cerebral blood flow. There is little research on the mechanism of this phenomenon, although much speculation about possible upregulation of individual inflammatory cytokines. Fever normally induces upregulation of heat shock proteins.
- **Several studies have attributed this to elevation of IL-17A during fever-induced inflammation. Release of IL-17A suppresses a region of the brain's cortex that has been linked to social behavioural deficits in mice. Injection of IL-17A had a beneficial effect on autism behaviour in mice.**
- **Paradox: it seems that IL-17A causes foetal developmental disorder with maternal immune activation…but IL-17A can also repair the damage caused during fever and inflammation.**
- **No attempt has been made to explain the paradox.**
- There is some indication that heat shock proteins may both augment and neutralise IL-17 signalling, but this is a largely unexplored research area, particularly with respect to autism.
- Sulphoraphane, a mitochondrial remedy, acts in part by upregulating heat shock proteins (HSPs), which are effective in correcting misfolded proteins found in autistic brains. It can also suppress IL-17 expression.

(Grzadzinski R, Autism Res, 2018; Good P. Neuropsychol Rev 2011; Calabrese V, J Neurosci Res, 2016; Singh K, CNS Neurol Disord Drug Targets, 2016; Liang J, Front Immunol, 2018; Mutlu ZS, J Exp Basic Med Sci, 2021; Reed MD, Nature, 2020; Al-Ayadhi LY, J Neuroinflammation, 2012; Mehler MF, Brain Res Rev, 2009; https://iancommunity.org/ssc/fever-effect-curious-phenomenon-autism)

# Mitochondrial therapies and ASDs

- Fasting or caloric restriction: No human studies.
- **Ketogenic diet:** showed significant improvement in CARS and ATEC scores and outperformed a gluten- and casein-free diet for improvement in cognition and sociability (El-Rashidy O, Metab Brain Dis, 2017; Evangeliou A, J Child Neurol, 2003). This suggests that it is the increased fat intake that is important.
- **Exercise**: A systematic review found that jogging, horseback riding, martial arts, swimming, yoga and dance can result in improvements to stereotypic behaviours, social-emotional functioning, cognition and attention in children aged up to 16 (Bremer E, Autism, 2016). Other studies found improvement in parent-perceived quality of life (Toscano CVA, Percept Mot Skills, 2018).
- Hyperbaric oxygen: Mixed results.
- Near infrared, hypothermia, pulsed EMFs: No human studies

# Mitochondrial remedies for which there is evidence of efficacy in ASDs (Annex G)

- B vitamins
- L-carnitine
- L-carnosine
- Magnesium
- Melatonin
- Omega 3 fatty acids
- Sulphoraphane
- Vitamin A *
- Vitamin D
- Zinc *
- Luteolin
- Resveratrol

Vitamins and minerals with *
All essential for mitochondrial function but in excess can be highly toxic.
Both mitochondrial deficiency and excess induce mitochondrial damage.

Flavonoids
A combination works best, as this is how they arise in nature. Their individual effects complement each other.

# Mitochondrial remedies in ASD: Observations

- Sulphoraphane has been found to lower IL-17A.
- There is now concern that mothers taking folic acid to prevent neural tube defects is giving rise to unmetabolised folic acid, which is a risk factor for autism.
- Instead, try methylfolate/5-methyltetrahydrofolate, folinic acid etc.

# Top 6 remedies with reasonable evidence of efficacy in humans

- L-carnitine: 50-100 mg/kg/day
- L-carnosine: 800 mg/day
- Melatonin up to 5mg at night
- Omega 3 fats: 200 - 1540 mg/day (but may need to give L-carnitine as well)
- Sulphoraphane: 50-150 µmol/day
- Vitamin D: 2000 IU/day

# Cell danger response (CDR)

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**https://naviauxlab.ucsd.edu/**

# CDR is not recognised by conventional medicine

- A PubMed search on ‘Cell danger response’ yielded 8 papers by Naviaux and 1 by an Italian team who mentioned it in passing at the end of the Discussion.
- So conventional medicine and academia don’t recognise the cell danger response as a medical phenomenon.
- So we need to be careful talking to other health professionals, who may never have heard of it!

# Background to Naviaux's work

- Naviaux quickly realised that complex chronic diseases were not linked to the start of cellular stress, but occurred during the healing phase. This finding is the key to understanding his work.
- He acknowledges that all the breakthroughs that have come from his lab in understanding complex chronic disorders have come from his early work in genetic forms of mitochondrial disease.
- From this he realised that healing from any injury requires both a mitochondrial reserve capacity and the ability to shift from one kind of mitochondrial function to another under times of stress or injury. These properties were missing in children with inherited forms of mitochondrial disease and are gradually lost with ageing.
- Others have also paved the way for his hypothesis: the polyvagal theory of Stephen Porges, the cellular stress response and damage-associated molecular patterns (DAMPs) that can initiate and perpetuate a *non-infectious* inflammatory response.

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# Metabolic features of the cell danger response

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This is Robert Naviaux's ground-breaking 2014 paper.

# Key points from Naviaux's papers: Initiation of the CDR

- **Mitochondria sense and respond to changes in the cellular environment,** which are translated into changes in mitochondrial structure and function.
- **When a threat (pathogen, toxin, etc) is detected, the cell danger response (CDR) is initiated.** This involves the **release of ATP into the circulation (extracellular ATP),** known as **purinergic signalling.**
- The **extracellular ATP acts as a damage-associated molecular pattern (DAMP)**. Other DAMPs include extracellular mitochondria and mtDNA. **This triggers innate and adaptive immune responses.**
- **Cellular ATP usage is inhibited, so that more will be available for extracellular danger signalling**. Many cellular processes, including methylation, are shut down. Mitochondrial fusion and biogenesis stop.
- Electrons are diverted from the ETC ('electron steal'), resulting in a drop in mitochondrial membrane potential, increased ROS production and dysregulated intracellular calcium.
- The cellular membrane hardens and develops patches of sphingolipids and cholesterol.

# Summary of Naviaux's 2014 paper (1)

- The cell danger response (CDR) is a cellular response to a threat (pathogen, toxin etc) involving the regulated release of purine and pyrimidine nucleotides such as ATP into the circulation (extracellular ATP) as a damage-associated molecular pattern (DAMP), a danger signal to other cells.
- ATP is known to be released in high concentrations after catastrophic disruption of the cell, as occurs in necrotic cell death. Extracellular ATP binds to P2 purinergic receptors.
- Both extracellular ATP and DNA act as DAMPs. DAMPs warn neighbouring cells and distant effector cells of danger and can trigger an immune response, activating microglia and inflammasome assembly. Cellular ATP usage is inhibited, so that more will be available for extra-cellular danger signalling. Consequently, the cell is a highly oxidising environment.
- Short term activation of purinergic receptors involve normal behaviour, including memory, feeding, locomotion and cognition. Chronic activation of purinergic receptors is found in a wide range of inflammatory diseases, including neurodegenerative disease, arthritis, depression, cancer, asthma.
- Cells accumulate debris as the CDR inhibits the normal release of exosomes (lipid nanovesicles containing cell debris) into the extracellular space as they could be hijacked by pathogens for growth.
- This has an effect on many body systems including the microbiome and can allow the accumulation of toxic metals, using up available antioxidants. Tryptophan metabolism is diverted from producing serotonin or melatonin, resulting in intense anxiety, depression and other mental health issues. [Typically hypervigilance, 'don't feel safe'.]

# Summary of Naviaux's 2014 paper (2)

- The CDR is initiated when mitochondria detect the diversion of electrons (known as 'electron steal') from the ETC. This is sensed as a voltage drop: a decrease in the electron flow available for oxidative phosphorylation. Mitochondrial electron flow therefore acts as a barometer of cellular health.
- Purinergic effectors such as ATP are co-released with neurotransmitters (glutamate, dopamine, serotonin) during membrane depolarisation at every synapse in which they have been studied and play key roles in activity-dependent synaptic remodelling. This led Naviaux to hypothesise that the CDR was maintained by purinergic signalling.
- Mitochondrial fusion-fission dynamics shift toward fission and mitophagy and away from fusion/biogenesis and mitochondria eventually fragment, leading to dysregulated intracellular calcium homeostasis and increased ROS production.
- Various processes are shut down, including methylation.
- Naviaux says that we should not talk about 'mitochondrial dysfunction' when mitochondria shift from energy production by OXPHOS to ROS production. This state is an adaptive and perfectly appropriate function of mitochondria, that is produced when cells come under stress. Mitochondria shift regularly and necessarily between these states throughout life.
- This changes the whole paradigm of diagnosis and treatment of chronic disease. Naviaux says that it is not the oxidative changes that should be targeted for therapy, but rather the metabolic conditions that create them.

# Key points from Naviaux's papers: Manifestations of the CDR

- **When the CDR is triggered**, our cellular priorities are reset to optimise survival. **We start to display sickness behaviour:** withdrawal from social contact, decreased speech, fragmented sleep, inflammation, aches and pains, gut microbiome changes, hypersensitivity to touch, sound and light.
- Naviaux describes children with autism being in a state of **primed hypersensitivity** as they encounter ever increasing amounts of environmental toxins with an underdeveloped detoxification system.
- With respect to autism, one of the conditions caused by activation of the CDR, gene studies conclude that each genetic cause of ASD must be treated differently. The CDR hypothesis suggests that just one mechanism might be at the root of all the different causes of chronic disease.

# Key points in Naviaux's papers: What happens when the CDR fails to resolve

- **In theory, if the danger (pathogen or toxin) is eliminated or neutralised, the CDR should be turned off**.
- **Naviaux believes that sometimes the CDR fails to resolve, even after the inciting agent has been removed.** It becomes stuck in a repeating loop that blocks healing. He likens this to **cellular post-traumatic stress disorder (PTSD)**.
- **He suggests that with the CDR stuck in the 'on' position, this is when chronic disease results. It could be autism or it could be T2D, CVD, etc.** Another example might be chronic inflammatory response syndrome (CIRS), as described by Dr. Richie Shoemaker, Desperation Medicine, and www.survivingmold.com.
- When Naviaux thinks this is due to a metabolic memory of the exposure, which is stored in the mitochondria.
- He believes that it is not the differences, but rather the similarities, that are shared by all chronic illness, that are important for understanding the root cause and hence the treatment.

# Failure of the CDR to resolve

- Sometimes the CDR is not resolved when the threat is removed. If healing is incomplete, cells can be left in a state of hyper- or hypo-responsiveness to new threats. We have seen this with inflammation or stress that cannot turn itself off without an intervention, so the concept is not new.
- Cells cannot heal if a significant fraction of ATP is exported for purposes of purinergic danger signalling instead of being kept in the cell for normal energy metabolism. This purinergic signalling plays the key role in sustaining the CDR and leads to chronic disease.
- Naviaux describes other studies that have shown that chronic changes in purinergic signalling alter pain perception and the processing of other sensory stimuli. He notes that this is not unlike a cellular form of post-traumatic stress disorder (PTSD) resulting in behaviour change after exposure to a transient, but serious stress.

# Implications for chronic disease

When the CDR fails to resolve, even after the inciting agent has been removed, chronic disease results, possibly as a result of the metabolic memory. A small selection includes:

- autism spectrum disorders (ASDs),
- attention deficit hyperactivity disorder (ADHD),
- food allergies,
- asthma,
- bipolar disorder,
- schizophrenia,
- post-traumatic stress disorder (PTSD),
- diabetes,
- kidney, liver and heart disease,
- cancer,
- Alzheimer and Parkinson diseases,

Naviaux's research has led him to the conclusion that it is not the differences, but rather the similarities, that are shared by all chronic illness, that are important for understanding the root cause and hence the treatment.

His research shows that the shared roots of chronic illness are blocks in the healing process itself, and these are caused by abnormal persistence of the cell danger response.

# When is mitochondrial dysfunction not dysfunction at all?

- Naviaux points out that the CDR gives the appearance of mitochondrial dysfunction, but is actually a normal, necessary, and highly regulated change in mitochondrial function from oxidative phosphorylation to cellular defence.
- This shift is needed to respond to a threat, and to heal after an injury. This programmed change in mitochondrial function is needed for innate immunity and inflammation, which in turn are required for establishing the adaptive immune response and healing.
- He has termed these 2 distinct mitochondrial functions M1 and M2.
- M2 mitochondria are devoted to OXPHOS and are anti-inflammatory. In contrast, M1 mitochondria are pro-inflammatory and are specialised for creating the oxidative shielding response for cellular defence by producing a pro-oxidative state and mobilising anti-viral and anti-microbial protection. M0 mitochondria also exist and are the 'metabolically uncommitted' mitochondria found in stem cells.
- Under the CDR, clearly the mitochondria must continue to produce some ATP, and the extent of continued OXPHOS depends upon the severity of the threat but can cease altogether if the survival of the cell is threatened.

# Naviaux's mouse trials

- **Hypothesis: autism results from failure to resolve the CDR; the CDR is sustained by purinergic signalling; therefore anti-purinergic therapy can improve autism.**
- First of all, he **demonstrated that abnormal persistence of extracellular ATP signalling could cause ASD-like behaviour and excitotoxicity**, leading to the death of Purkinje cells in the cerebellum.
- Naviaux then trialled **suramin, a well-studied anti-purinergic agent, which showed improved behavioural and metabolic features in the mice**, including decreased excitoxicity and prevention of Purkinje cell death. Suramin treatment improved 94% of the abnormal biochemical pathways in the MIA mouse model and 100% of the pathways disturbed in the Fragile X mouse model.
- Suramin has been used for decades in the treatment of African Sleeping Sickness (Trypanosomiasis).
- Naviaux carried out 3 trials using the Fragile X genetic model of ASD and the maternal immune activation (MIA) model involving maternal injection with a virus.

(Naviaux R, 2013, 2014 and 2015)

# What is suramin

Developed in 1916 by German dye manufacturers Frederich Bayer and Co., Bayer 205 (later renamed suramin) was found to be effective against parasitic trypanosomes responsible for African sleeping sickness (trypanosomiasis).

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RESEARCH ARTICLE

**Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial**

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- Now Naviaux carried out an **RCT in autistic boys, who received just <u>one</u> i/v infusion of low dose suramin** or saline.
- **Suramin improved all the core symptoms of ASDs with just 1 dose.**
- **Two children who were previously non-verbal spoke their first sentences. All of the children who received suramin experienced catch-up development,** with one child advancing through 3 years of schoolwork in 3 weeks.
- **The peak benefit from the single dose of suramin occurred after 3 weeks, then decreased.**

(Naviaux RK, Ann Clin Transl Neurol, 2017)

# Naviaux 2017: Results of the RCT

- During the first few weeks, the CDR was reduced and more normal metabolism restored. Purine metabolism was the most improved pathway, with increased healthy purines such as an activator of AMPK.
- ADOS scores (the standardised diagnostic test for autism) improved significantly in the treatment group only. There were also improvements in communication, language, social interaction and restricted or repetitive behaviours.
- The peak benefit from the single dose of suramin occurred after 3 weeks, then decreased. Measurements gradually returned to baseline after 5-8 weeks. [This suggests that the conditions giving rise to the CDR still exist: toxins? infection?]
- Low dose suramin proved to be safe; the only adverse effect was temporary rash.
- Subsequent studies by others have found that suramin prevents Zika, Ebola, Chikungunya, Coxsackievirus A16, and Enterovirus A71.

# Naviaux on Anti-Purinergic Therapy

- Over 30 metabolic abnormalities have been described in ASD over the past 60 years; all are known markers of the CDR.
- Naviaux also found that each of the common genes known to increase the risk of ASD can be shown to play a role in CDR signalling or maintenance. But while most gene studies conclude that each genetic cause of ASD must be treated differently, the CDR hypothesis suggests that one mechanism—a unified cellular response—might be at the root of all the different causes of autism.
- Evidence of purinergic signalling abnormalities have been found in children with ASD in a 2012 study and purinergic signalling has been shown to regulate a number of the cellular comorbidities and functional abnormalities associated with ASD.
- Naviaux describes using the antipurinergic suramin to treat a misfiring CDR as 'molecular armistice therapy' because it sends a signal that 'the war is over'. This decreases losses of ATP through stress-gated membrane channels, and decreases purinergic autocrine and paracrine signalling of danger so cells and mitochondria can return to peacetime metabolism needed for healing and development. See Naviaux video at (https://www.youtube.com/watch?v=zIdUufy8Lk)

# Independent corroboration and CDR developments

- A 2015 review looking at purinergic signalling in psychiatric disorders confirmed that **extracellular ATP acts as a neurotransmitter, and that purinergic signalling may explain its impact on neuronal activity.** The authors show how mitochondrial and purinergic dysfunction contribute to mental illnesses such as schizophrenia, bipolar disorder, autism spectrum disorder (ASD), depression and addiction. They also report that changes in mitochondrial bioenergetics and purinergic signalling via adenosine receptors have been proposed as explanations for the neuroprotective and anti-seizure effects of ketogenic diets. (Lindberg D, Curr Mol Med, 2015)
- Naviaux is running a new study, called the **Suramin Autism Treatment 2 (SAT2) trial**, which was due to start spring 2021. He also wants to conduct clinical trials in ME/CFS and primary forms of mitochondrial disease.
- He is also writing a book entitled '**A Second Book of Medicine**', **intended to treat the epidemic of chronic disease from the perspective of the CDR**. It will involve a new class of medicines to help doctors adjust the set-point of the CDR so it is no longer hypersensitive to chemicals and other threats that are below the threshold required to cause obvious harm. The new medicines will help send the chemical message that the 'war is over', allowing the patient struggling with a chronic illness to start to heal. (Naviaux RK, Mitochondrion, 2020 and personal correspondence)

# What about IL-17A?

- **We have already shown that IL-17A can be elevated in autism, particularly where the mother had immune activation**. 'Our study further suggests that blockade of IL-17A/IL-17 receptor signaling may be beneficial in the children with ASD.' (Nadeem A, Brain Behav Immun, 2017)
- Studies have shown that as well as being an antipurinergic drug, **suramin can also lower IL-17A**. Nevertheless, 'The mechanism of action for suramin is unclear' (Wikipedia). *
- Does this mean that suramin will not work in ASDs not derived from maternal immune activation? Note the paradox that we saw earlier, where children with autism associated with maternal immune activation had elevated IL-17A, but further elevation in fever could also improve cognitive and behavioural symptoms.
- In experimental arthritis, suramin strongly inhibited IL-17-generated cytokines (Fan ZD, Sci Rep, 2016)
- In experimental asthma, allergen challenge induced increased ATP secretion, which correlated with IL-17 production. When ATP secretion was blocked with suramin, Th17-type responses were reduced significantly (Zhang F, J Immunol Res, 2017).

# Timing of vaccinations to reduce autism risk

- Naviaux makes the point that since any infection can initiate the CDR, this means it can also be triggered following vaccination.
- And since the severity of the CDR is proportional to the severity of the cellular attack, it follows that the body is more likely to resolve the CDR and return to normal health if vaccinations are staggered instead of giving a large number at once at an age when the immune system and neural circuits are at their most fragile.
- Naviaux speculates that the CDR may be more difficult to resolve when it is triggered *in utero* or at a very young age.
- Naviaux, autism and suramin:

  https://www.youtube.com/watch? v=zIdUufy8Lks

  http://naviauxlab.ucsd.edu/science-item/autism-research/

# Naviaux's antecedents: Polyvagal theory

- Autonomic nervous system: sympathetic and parasympathetic. Sympathetic = fight or flight response. Parasympathetic = the calming down and healing response.
- But not always. If the environment is perceived as unsafe, the para-sympathetic nervous system goes into 'death-feigning' mode i.e. the freeze response. This is when the body actually shuts down, cf animals who 'play dead' to avoid predators. This is the mitochondrial 'dauer' response seen in CFS.
- Relating this to the vagal nerve, the freeze response is activated by the dorsal side (the ventral side is for healing). The ventral side is switched off by the CDR, vagal tone is down and there is low heart rate variability (the test of vagal tone).
- The stages of the CDR appear to correlate with equivalent changes in vagus nerve expression.
- Stimulating the vagus nerve has been shown to improve a wide variety of conditions, including seizures, heart failure, ischaemia-reperfusion injury, stroke, migraine, neurodegeneration, PD, inflammation, PTSD, depression, anxiety, endocrine disorders, obesity and many other.

(Porges SW, Cleve Clin J Med, 2009)

# Naviaux's antecedents: Cellular stress response

- When the cell is stressed, it first goes into defensive mode for protection and to aid recovery. If this fails, then the cell activates apoptosis pathways.
- This type of cellular stress has been seen in many chronic diseases, including T2D, ischaemia, atherosclerosis, cancer and neurodegenerative disorders.
- Typical protective responses include upregulating antioxidant enzymes, heat shock protein production, the unfolded protein response and the DNA damage response.
- Heat shock proteins (HSPs) shut down general protein transcription and translation to conserve energy. They also inhibit the apoptotic pathways and upregulate pro-survival mechanisms.
- The unfolded protein response (UPR): can be induced by many stimuli, including toxins, resulting in protein damage; the unfolded proteins accumulate in the endoplasmic reticulum. Misfolded proteins are found in many neurodegenerative diseases. To counter the damage, cells upregulate chaperone proteins to help refold the proteins and secrete growth factors to promote cell survival.
- The DNA damage response: when toxins, radiation or drugs have caused single or double DNA strand breaks. Single strand breaks undergo nucleotide excision repair using the intact strand as a template; double strand breaks require more complex repair mechanism (non-homologous end joining and homologous recombination).
- Examples: In Parkinson's disease, activation of heat shock proteins seen through over-expression of alpha-synuclein in dopaminergic neurons; both HSPs and the UPR have been found in animal models of myocardial infarction and cancer.



(Fulda S, Int J Cell Biol, 2010)

# Naviaux's 2016 paper: a metabolomics study on CFS

## Metabolic features of chronic fatigue syndrome

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- Naviaux showed that CFS patients had abnormalities in 20 metabolic pathways, most of which indicated a hypo-metabolic syndrome, showing a diagnostic accuracy of around 95%.

- 9 of these pathways related to the CDR, with the down-regulation suggesting post-exposure adaptation or a mitocellular hormesis response to pathologically persistent or recurrent cell danger signalling.

# More on P2Y receptors and purinergic signalling

- Extracellular ATP binds to P2Y receptors. P2Y receptors are a family of purinergic G protein-coupled membrane receptors, present in almost all human tissue, which are stimulated by nucleotides (purines and pyramidines) such as ATP and ADP. There are 8 different P2Y receptors.
- In the brain, P2Y receptors are largely involved in presynaptic activities, as well as mediating long-term (trophic) signalling in cell proliferation, differentiation and death during development and regeneration and participating in neuron-glial interactions. Purinergic signalling is involved in control of cerebral vascular tone and remodelling and has been implicated in learning and memory, locomotor and feeding behaviour and sleep. There is increasing interest in the involvement of purinergic signalling in the pathophysiology of the ischaemia, epilepsy, neurodegenerative diseases, neuropsychiatric and mood disorders and cancer. (Burnstock G, Adv Exp Med Biol, 2020)
- By regulating flow of calcium and potassium ions, P2Y receptors mediate various responses including vasodilatation, blood clotting, heart failure and the immune response (Birkenfeld AL, Pharmacol Ther, 2019).
- Several drugs target P2Y receptors, including the anti-platelet clopidogrel. Interestingly, a biological exposure is known to turn on coagulation pathways.
- In 2017, a search of the keyword ‘purinergic’ among US intervention trials returned 418 studies. Currently, over 90% of these studies are focused on platelets and heart disease.
- There appears to be broad involvement of purinergic signalling in nearly every chronic disease in which it has been studied, as shown by the work of Geoffrey Burnstock, who coined the term ‘purinergic signalling’, and is referenced extensively by Naviaux.

# Extracellular mitochondria

- Extracellular mitochondria can be found free, enclosed by a membrane (inside platelets or vesicles) or as cell-free circulating mitochondrial DNA.
- All forms of extracellular mitochondria can induce paracrine or endocrine responses. These are forms of signalling: paracrine signalling involves communication with nearby cells, i.e. over a short distance, whereas endocrine signalling involving hormones is used when cells need to transmit signals over long distances.
- Extracellular mitochondria could act as regenerative factors or pro-inflammatory activators, particularly in the activation of the immune system. They have potential in disease diagnostics and in the treatment of cardiac, neurological and metabolic diseases. Their effects depend on the mitochondrial state or the form in which it is present, either as a whole functional structure as fragments or only as mitochondrial DNA. (Miliotis S, Mitochondrion. 2019; Torralba D, Front Cell Dev Biol. 2016)
- Extracellular mitochondria or their components have also been found to transfer between cells to support OXPHOS in recipient cells. In the CNS, this facility is also being explored as treatment in spinal cord injury, Parkinson's disease, schizophrenia and ischaemic stroke. (Nakamura Y, Park JH, Hayakawa K. Therapeutic use of extracellular mitochondria in CNS injury and disease. Exp Neurol. 2020)

# Extracellular mtDNA

- Chronic stress may cause cellular damage and mitochondrial dysfunction, potentially leading to the release of mitochondrial DNA (mtDNA) into the bloodstream. Major depressive disorder has been associated with an increased amount of mtDNA in blood leukocytes.
- The authors, unconnected with Naviaux, found that suicide attempters may have elevated plasma levels of mtDNA, which are related to impaired HPA-axis negative feedback (i.e. a negative feedback loop has become a positive feedback loop). This is consistent with increased cellular or mitochondrial damage. (Lindqvist D, Transl Psychiatry, 2016)
- Another study (also unconnected with Naviaux) found that exposure to psychological stress increases serum circulating cell-free mtDNA levels up to 3-fold in healthy midlife adults. In vitro studies showed that glucocorticoid signalling as a consequence of psychological stress was sufficient to induce the mtDNA extrusion. (Trumpff C, Psychoneuroendocrinology, 2019)