Might cholesterol sulfate deficiency contribute to the development of autistic spectrum disorder?

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ABSTRACT

Autism is a condition characterized by impaired cognitive and social skills, often associated with compromised immune function. There has been considerable concern recently that the incidence of autism is alarmingly on the rise, especially in Western nations, and environmental factors are increasingly suspected to play a role. In this paper, we propose a novel hypothesis for a principle cause of autism, namely insufficient supply of cholesterol sulfate to the fetus during gestation

and the infant postnatally. We hypothesize that main contributory factors are insufficient sun exposure and insufficient dietary sulfur, for both the mother and the affected child. A novel contribution is the theory that endothelial nitric oxide synthase produces not only nitric oxide but also sulfate, and that sulfate production is stimulated by sunlight. We further hypothesize that the sulfur shortage manifests as an impaired immune response, including an increased susceptibility to eczema and asthma. Proposed corrective measures involve increased dietary sulfur intake for both the mother and the child, and increased sun exposure.

1. INTRODUCTION

Autism, and, more broadly, autistic spectrum disorder (ASD), is a condition characterized by impaired cognitive and social skills [1], along with a compromised immune function [2].
It can now no longer be denied that the incidence of ASD is alarmingly on the rise in the U.S. [3]. While considerable research effort has been devoted to trying to uncover the cause(s) of autism, thus far no definitive answer seems available from the research literature. Autism is hypothesized to have a strong genetic component, due in part to the high concordance seen in identical twins compared with fraternal twins [4]. Much of the research has been devoted to finding associated genetic risk factors [5]. Although some encouraging links have been found, such as, for example with observed genetic mutations in neurexin [6] and neuroligin [7], no single gene variant has been found that can account for more than 2% of the affected population. The fact that ASD rates have been rapidly increasing over the last few decades strongly points to an environmental component [8].
A plausible explanation for identical twin concordance could also be a deficiency in the supply of a critical nutrient from the mother. Identical twins share a single placenta 50% of the time, and this could cause a further depletion of a scarce resource, beyond the additional load imposed by two fetuses having to share the limited supply. In fact, just being a twin is a strong risk factor for autism [9], as fraternal twins (whose genetic closeness is identical to that of siblings) have a substantially increased concordance in ASD compared to siblings. This logically results from the doubled nutritional requirement of two fetuses.

What might such a hypothesized critical nutrient be? Smith-Lemli-Opitz syndrome, characterized by a defect in the conversion of 7-dihydrocholesterol to cholesterol [10] is almost universally associated with severe autism, strongly suggesting that cholesterol deficiency is related to autism [11]. Cholesterol is present in all cellular membranes, where it is vital for their proper structure and function [12]. Notably, cholesterol represents a key component of cellular membranes' lipid rafts, which are cholesterol- and sphingolipid-enriched membrane microdomains that function as platforms to concentrate and segregate proteins within the plane of the bilayer [13]. The brain houses 25% of the body's cholesterol, with only 2% of the mass. In particular, de-novo cholesterol is essential in the central nervous system for both synaptic fusion [14] and myelin membrane growth [15]. A strong suggestion that defective synaptic transmission is associated with ASD comes from the known link between some cases of autism and genetic defects in neurexin and neuroligin, two proteins that are intimately related to neurotransmitter transport across synapses [16].

We thus decided to investigate the mechanism by which the mother supplies cholesterol to the fetus. Our research revealed interesting insight that led us to hypothesize that cholesterol sulfate, which can cross the placental barrier much more readily than cholesterol, is the main source of cholesterol from the mother to the fetus. Therefore, maternal sulfur deficiency might be a key contributor to ASD in the child.

We further propose, more specifically, that endothelial nitric oxide synthase (eNOS) plays a central role in the pathology of ASD.

We have developed the novel hypothesis that eNOS, in addition to its role in producing small amounts of nitric oxide (NO), has a more significant but heretofore overlooked role as a major supplier of sulfate to the extracellular matrix proteins throughout the body.

2. Cholesterol Sulfate Supply to the Fetus

The mechanism by which the mother supplies cholesterol to the fetus is even today poorly understood, and given the importance of cholesterol to the fetal nervous system, this mechanism deserves further study. Direct cholesterol transport across the placenta is challenging, given that the placental barrier is impermeable to low-density lipoprotein (LDL), and it had long been assumed that the fetus supplies its own cholesterol de novo [17]. However, this cannot be true, as children suffering from Smith-Lemli-Opitz syndrome develop relatively normally in the womb. Mothers who have high serum levels of cholesterol give birth to infants with low serum cholesterol, a seeming contradiction, which, however, can be easily explained by a proposal that the normal mechanism of cholesterol delivery from the mother to the fetus is achieved by sulfating the molecule. This implies that women with high cholesterol have a reduced supply of cholesterol sulfate, and this could even be the reason for their high cholesterol. This theory gains significant support from the observation that the placental villi are normally highly enriched in cholesterol sulfate, especially as the pregnancy approaches full term [18]. Furthermore, measurements have shown that the mother's serum cholesterol sulfate level is elevated already in the first term and steadily rises throughout the pregnancy. The concentration of cholesterol sulfate in the placental villi in the third trimester was measured as 23.8 pmol/mg dry mass, up

sharply from 3.93 in the first trimester, and to be contrasted with the value of only 1.56 for blood serum levels of a non-pregnant woman.

Cholesterol sulfate is an intriguing molecule whose purpose in human physiology remains poorly understood [19]. It is synthesized in abundance in the skin by melanocytes and fibroblasts, as well as by the liver for incorporation into bile acids, and by RBCs and platelets. It circulates in the bloodstream at a relatively high concentration of 150-300 micrograms per 100 milliliters, but is highly transient with rapid turnover. Its important role in decapitation of sperm makes it essential for fertilization [20]. Its rate of inter-membrane exchange is approximately ten times faster than that for cholesterol [21]. An important feature of cholesterol sulfate is that it is amphiphilic, due to its negative charge, and thus it can travel freely in the bloodstream rather than being packaged up inside an LDL particle for delivery. Its amphiphilic nature would allow it to cross the placental barrier much more readily than cholesterol.

Cholesterol sulfate co-locates with profilaggrin in the granular layer of the epidermis. In fact, it has been confirmed that cholesterol sulfate stimulates gene transcription of profilaggrin by binding to retinoic acid receptor-related orphan receptor alpha (RORα). Factors that interfere with RORα or with sulfatase lead to an 80% reduction in profilaggrin synthesis [22]. Profilaggrin is the precursor to filaggrin, which plays a crucial role in the barrier, keeping microbes out and preventing water loss [23]. A defective profilaggrin gene is associated with asthma [24], and asthmatic mothers have double the risk of giving birth to autistic children [25]. Furthermore, autism itself is associated with asthma [26], which suggests that the cholesterol sulfate deficiency in the mother has translated to a similar deficiency in the child. Defective filaggrin is also

associated with eczema [27], and a recent study has demonstrated a link between autism and eczema [28].

We hypothesize that the sulfate anion in cholesterol sulfate is also extremely important to fetal development, in addition to the cholesterol. Fetal tissues have a limited capacity to produce sulfate, and therefore rely on sulfate obtained from the maternal circulation [29]. Sulfate supply is necessary for adequate sulfation of glycoproteins, glycolipids, and glycosaminoglycans, such as heparan sulfate and chondroitin sulfate, which form crucial components of the extracellular matrix proteins throughout the body [30]. Heparan sulfate proteoglycans are abundant in basement membranes in the placenta, where they influence cellular signaling and interact with growth factors and cell receptors [31]. Sulfate is also essential for the detoxification of xenobiotics and commonly administered drugs like acetaminophen (paracetamol) [32]. A possible link has been found between acetaminophen and both autism and asthma [33].

A little known fact is that the vitamin D3 present in human milk is sulfated. In fact, vitamin D3 produced in the skin from cholesterol upon sun exposure is sulfated, and, like cholesterol sulfate, it can travel freely in the blood [34]. Both human milk and colostrum contain a significant amount of sulfur, with colostrum being substantially enriched (10.2 mmol/liter) compared to human milk: (4.3 mmol/liter) ($p < 0.001$). [35]. In this fashion, continued sulfate supply is assured for the newborn infant.

3. Estrogen and 5-Dehydroepioandrosterone Transport Mechanisms Involve Sulfate

It has recently been hypothesized that ASD is a manifestation of an extreme form of the “male brain,” [36] favoring systemizing over empathizing thought processes [37]. Such a “super-male”

phenotype would likely arise out of insufficient estrogen supply from the mother during embryonic development.

Further support for the concept of estrogen deficiency as a feature of ASD comes from the observation that ASD is associated with reduced cortical thickness in bones [38]. Bone depends critically upon estrogen for its formation, and it has been shown that estrone sulfate is a much more efficient supplier of estrogen to bone than is estrogen itself [39]. Similar to the situation with cholesterol sulfate, adding a sulfate anion to estrogen renders it amphiphilic, allowing it to be transported freely through the bloodstream. It seems plausible that sulfation would be the mechanism by which estrogen would be transported across the placenta, for the same reason that cholesterol sulfate is the main supplier of cholesterol. Sulfate deficiency in the mother would thus translate into estrogen deficiency in the developing fetus.

In addition to its role in profilaggrin synthesis, RORα transcriptionally regulates the enzyme aromatase, which converts testosterone to estrogen. RORα has been shown to be deficient in the frontal cortex in association with ASD [40]. RORα is stimulated by estrogen, so estrogen deficiency would explain its reduced presence in the autistic brain. Furthermore, this feedback loop (estrogen stimulates further production of estrogen from testosterone) would magnify the deficit initiated by the mother's inadequate supply of estrone sulfate, leading to the "super-male" phenotype associated with ASD.

The most abundant circulating steroid in humans is 5-dehydroepioandrosterone (DHEA), which is synthesized from cholesterol and secreted by the adrenal glands. It serves as a precursor to both estrogen and testosterone. DHEA exists in both a sulfated and an unsulfated form, and

studies have shown that the sulfated form is depleted in association with ASD [41,42], further evidence for sulfate deficiency as a causative factor in ASD.

In a prospective study [43], individuals diagnosed with ASD were evaluated with regard to a number of transsulfuration metabolites, including reduced and oxidized glutathione, cysteine, taurine, and sulfate. Study subjects had significantly reduced levels of every metabolite tested except oxidized glutathione, which was significantly elevated. The serum level of plasma total sulfate was less than half of that found in the control group, a result that was highly significant ($p < 0.0001$). The most prominent abnormality was the level of plasma free sulfate, which was only 33% of the mean control value. The fact that glutathione was found in excess in the oxidized state indicates increased oxidative stress.

4. Digestive System Dysfunction and Opioids

Autism is often associated with digestive system dysfunction, and a treatment program that has gained popularity is to introduce a gluten-free and casein-free diet [44]. Anecdotally, this dietary change has been demonstrated to improve symptoms in some cases. The opioid-excess theory of autism suggests that excessive levels of incompletely metabolized peptides pass through the intestinal and blood-brain barrier, acting as opiates on the central nervous system [45]. Peptides found in gluten and casein, if they can penetrate the gut lining and the blood brain barrier, can act like opiates such as morphine by binding to brain opiate receptors [46]. These are referred to as “exorphins” to distinguish them from endorphins, which are synthesized in vivo. It has been

confirmed through multiple studies that autistic children have elevated levels of opioids, both in the cerebral spinal fluid and in the peripheral blood mononuclear cells [45].

A reduction in the activity of certain digestive hormones, such as cholecystokinin and gastrin, has been found to be associated with a deficit in sulfation ability [47]. The mucins in the gut wall are sulfated glycoproteins, and reduced levels of sulfation have been associated with gut dysfunction and an increase in permeability [48]. Abnormalities in the structure of the intestinal wall and the protective mucus coat due to sulfate deficiency will lead to enhanced passage of peptides. Furthermore, low sulfate availability greatly reduces the capacity to detoxify amines and phenols. While attempts to supply sulfur-containing supplements such as cysteine have been unsuccessful, anecodotal evidence suggests improvements in behavior and physiology in some people following epsom salt treatments [47].

Autistic children have exhibited increased excretion of proteins in urine, along with high urinary sulfate, implying that the resorption step in the proximal tubule is defective [49]. This could be due in part to a deficiency in vitamin D3, which plays an important regulatory role in sulfate metabolism [50].

5. A Novel Role for eNOS

The nitric oxide synthases (NOS's) have been well studied since their discovery in 1987 [51], yet they are still not fully understood. Three related forms have been identified: neural NOS (nNOS),

endothelial NOS (eNOS), and inducible NOS (iNOS) [52]. Both nNOS and eNOS are constitutional, whereas iNOS is inducible based on certain environmental conditions, e.g., lipopolysaccharide (LPS) exposure subsequent to a microbial invasion. Another interesting distinction is that nNOS and eNOS require calcium influx to be activated, whereas iNOS does not. We propose here the novel hypothesis that eNOS is a dual-purpose enzyme, and that, in many cells, its main purpose is to produce sulfate rather than nitrate (the ultimate product from nitric oxide).

It has been confirmed, at least for piglets, that eNOS is present in substantial amounts in endothelial cells lining the lung vasculature at birth, and it has been suggested that it plays a crucial role in the adaptation to extra-uterine life [53,54]. There is an interesting alignment between cell types that synthesize eNOS and cell types that produce cholesterol sulfate [19, 55,56,57]. These include keratinocytes and epithelial cells in the epidermis, endothelial cells lining the blood vessels, and platelets and RBCs in the bloodstream. RBCs in particular have presented a puzzle to researchers, as they clearly contain substantial amounts of eNOS, which shows up mainly in the inner leaflet of the plasma membrane [57]. Yet RBCs maintain an extremely low level of the substrate, L-arginine, actively keeping it out and destroying it with L-arginase if trace amounts appear in the cytoplasm. Furthermore, hemoglobin is an excellent NO scavenger, and accumulations of NO bound to hemoglobin would interfere with oxygen transport, much like carbon monoxide. This is clearly a detrimental outcome to be avoided.

The eNOS molecule contains a heme group and a flavin group. It readily forms a dimer structure, with a fluid-filled cavity created between the two monomers. A zinc atom is situated in the center of the cavity, where it creates a positive charge field that attracts four sulfur atoms

associated with four cysteine molecules [58]. In the absence of L-arginine substrate, eNOS produces superoxide, O_2^-, instead of NO [59]. The flavins respond to UV exposure from sunlight by releasing electrons, which are then absorbed by oxygen molecules associated with the iron in the heme group of the opposing monomer. The zinc atom's charge would logically draw O_2^- into the cavity, where it can bind with a sulfur atom from one of the cysteine molecules to form $SO_2^{-\cdot}$. A second O_2^- supplied by the other monomer would then complete a sulfate anion. The cysteine molecule could be restored with a sulfur atom from glutathione via glutathione S-transferase, a ubiquitous enzyme known to bind to eNOS [60].

We hypothesize that the sulfate so formed, in most cases, reacts with cholesterol via a sulfotransferase to form cholesterol sulfate, which is then released into the bloodstream. The rapid turnover of cholesterol sulfate dictates that it will quickly find a home in the membrane of a neighboring cell, such as a leukocyte, an endothelial cell, a myocyte, etc. Cells in the epidermis would continuously resupply cholesterol sulfate to the granular layer, to maintain the barrier function.

In addition to RBCs and platelets, mast cells also synthesize eNOS. While they are not known to synthesize cholesterol sulfate, they are the major suppliers of heparin. Heparin is the most highly sulfated molecule known to biology, and hence would be a logical target for any sulfate synthesized by eNOS in mast cells.

6. DISCUSSION

Autism is a disorder affecting cognitive and social skills that has severe implications on the ability of the affected individual to lead a productive and independent life. The alarming increase

in the incidence of ASD in the last decade suggests that, while genetic factors are contributory, environmental triggers must also play a decisive role. In this paper, we argue that ASD is the result of a "perfect storm," a confluence of several factors including inadequate nutrition and insufficient sun exposure. The damage begins in utero, due to the mother's inability to supply adequate cholesterol sulfate to the developing fetus. Postnatally, further damage accumulates, due to aggressive use of sunscreen, avoidance of sun exposure and continued dietary sulfur deficiency that collectively lead to further damage to the child's brain.

Studies on cholesterol supply from the mother to the fetus have led us to hypothesize that sulfation plays a critical role in penetrating the placental barrier, and we argue that this is likely true for estrogen as well as for cholesterol. The mother's sulfate deficiency plausibly leads to a deficiency in cholesterol, estrogen, and DHEA in the fetal brain. This in turn results in defective brain development in the infant, and an impaired ability to process sensory inputs and transmit neural signals.

A serum deficiency in trans-sulfuration metabolites, particularly sulfate, has been amply demonstrated in association with ASD. Sulfate deficiency results in insufficient ionic buffering in the bloodstream, with grossly inadequate sulfation of the extracellular matrix proteins that are essential for proper development of the fetal nervous system and immune system. The association of both asthma and eczema with ASD can be explained as an inadequate supply of filaggrin, due to the fact that cholesterol sulfate in the epidermis stimulates the production of profilaggrin, its precursor. Sulfate deficiency is also associated with the digestive system dysfunction frequently found in autistic children, allowing peptides to infiltrate the blood serum as a consequence of incomplete digestion of gluten and casein. A novel contribution is the

proposed role for eNOS in synthesizing sulfate upon exposure to sunlight, and we argue that eNOS is a major supplier of sulfate both to the epidermis and the blood stream.

If our theory is correct, then it should be relatively easy and very cost-effective to implement a solution to the problem. Both women of childbearing age and children should be encouraged to consume foods that are rich in sulfur and to spend on sunny days considerable time outdoors without sunscreen, below the threshold dose for erythema.

CONFLICT OF INTEREST STATEMENT

The authors claim no conflicts of interest.

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