Brain carnitine deficiency causes nonsyndromic autism with an extreme male bias: A hypothesis

Arthur L. Beaudet

Could 10–20% of autism be prevented? We hypothesize that nonsyndromic or ''essential'' autism involves extreme male bias in infants who are genetically normal, but they develop deficiency of carnitine and perhaps other nutrients in the brain causing autism that may be amenable to early reversal and prevention. That brain carnitine deficiency might cause autism is suggested by reports of severe carnitine deficiency in autism and by evidence that *TMLHE* deficiency – a defect in carnitine biosynthesis – is a risk factor for autism. A gene on the X chromosome (*SLC6A14*) likely escapes random X-inactivation (a mixed epigenetic and genetic regulation) and could limit carnitine transport across the blood-brain barrier in boys compared to girls. A mixed, common gene variant-environment hypothesis is proposed with diet, minor illnesses, microbiome, and drugs as possible risk modifiers. The hypothesis can be tested using animal models and by a trial of carnitine supplementation in siblings of probands. Perhaps the lack of any Recommended Dietary Allowance for carnitine in infants should be reviewed.

Also see the video abstract here: https://youtu.be/BuRH_jSjX5Y

Keywords:
autism; blood-brain barrier; carnitine; diet; epigenetic; gene-environment; inborn errors of metabolism; microbiome; sex ratio

Additional supporting information may be found in the online version of this article at the publisher's web-site.

DOI 10.1002/bies.201700012

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Abbreviations:
ADHD, attention-deficit/hyperactivity disorder; **BBB,** blood-brain barrier; **BSRC,** Baby Siblings Research Consortium; **CNV,** copy number variant; **DHA,** docosahexaenoic acid; **GWAS,** genome-wide association study; **NoMeND autism,** non-Mendelian non-dysmorphic autism; **PCSD,** primary systemic carnitine deficiency; **PUFA,** polyunsaturated fatty acid; **TML,** trimethyllysine.

Introduction and hypothesis

Understanding the biochemical basis of a rare genetic disorder sometimes sheds light on the processes underlying a more common disease. The discovery of a not so rare inborn error of carnitine biosynthesis (deficiency of the X-linked *TMLHE* gene) may prove to be such a case. This deficiency, discovered in males with non-dysmorphic autism [1], prevents the synthesis of carnitine from trimethyllysine. Although 1 in 350 males (estimated ~460,000 males in USA) have *TMLHE* deficiency, only about 3% of these males develop autism, with most of the remainder becoming healthy adults; furthermore, less than 1% of autistic males have *TMLHE* deficiency. Nevertheless, we believe there are compelling reasons to think that brain deficiency of carnitine and perhaps other micronutrients such as essential polyunsaturated fatty acids (PUFA) can cause autism with an extreme male bias, and that 10–20% of cases of autism could be prevented by changes in infant nutrition.

The varied landscape of autism spectrum disorders versus "essential" autism

During the 1990s and earlier, autism was thought to be caused primarily by common variants [2, 3], but more

recently the roles of de novo copy number variants (CNVs) [4] and de novo point mutations [5], rare, individually pathogenic variants have received greater attention [6]. There are now hundreds of genes of varied functions implicated in the etiology of autism (https://sfari.org/resources/sfari-gene), with no one gene accounting for more than a tiny fraction of cases.

The spectrum of phenotypes described under the autism umbrella is equally heterogeneous, ranging from very severe syndromes with congenital malformations, intellectual disability, abnormal brain structure, and dysmorphic features, to children who appear normal but are socially impaired, may or may not have low IQ, and may fit previous definitions of Asperger syndrome (Fig. 1). This milder group is overwhelmingly male, and many publications report extremely high male/female ratios in this subgroup of 8/1 or higher (Table 1). One study [7] of 94 patients with autism found that "the total study group had a male to female ratio of 4.2/1; the morphologically normal subgroup, defined on the basis of a normal physical examination, had a sex ratio of 7.5/1 and the normal subgroup, defined on the basis of both a normal physical examination and a structurally normal brain by MRI, had a 23/1 sex ratio."

Could this milder group, with its marked male bias, form a subset of autism that has a relatively homogeneous pathophysiology or etiology? We might describe this milder phenotype as "essential" autism [7] or non-Mendelian, non-dysmorphic (NoMeND) autism with a normal physical and neurological examination. We occasionally use the term nonsyndromic or essential autism to mean that there is no known genetic abnormality or syndromic diagnosis. Unlike children with syndromic autism who are often dysmorphic, NoMeND by definition would exclude these children, as well as those with microcephaly or short stature at any age, macrocephaly at or before 3 months of age, severe hyper- or hypotonia, ataxia, abnormal reflexes, abnormal gait, prematurity, congenital malformations, dysmorphic features, or onset before 6 months of age. These non-dysmorphic children are sometimes described as beautiful. It is likely but not certain that NoMeND autism is associated with regression, especially if acquisition of a social smile at 6–8 months of age and subsequent loss is defined as regression. Regression has been reported in a male with *TMLHE* deficiency and very low plasma carnitine [8]. Epilepsy would be less common than with Mendelian autism, but it is uncertain if epilepsy should be reason for exclusion from the NoMeND group. The hypothesized etiology would exclude highly penetrant Mendelian mutations (e.g. deletion 16p11.2 or loss-of-function mutations in *CHD8*) but likely include common genetic carrier states and polymorphisms, diet, minor illnesses, microbiome changes, and environmental factors. *TMLHE* deficiency is low penetrance and would not exclude NoMeND autism.

Non-Mendelian, non-dysmorphic (NoMeND) autism, and the brain carnitine deficiency hypothesis

We posit that NoMeND autism patients suffer carnitine deficiency in the mitochondria in the brain, perhaps in glia or neurons including possibly at the synapse. (Brain deficiency of other micronutrients may contribute, as discussed below). We hypothesize that this brain carnitine deficiency disturbs neurodevelopment, perhaps specifically neurogenesis [9] or synaptic development [10]. Carnitine deficiency might simply reduce mitochondrial copy number generally [11]. Although the focus here is on carnitine deficiency as a primary form of pathogenesis, the same pathophysiology could act as a modifier for Mendelian or other forms of autism.

Lombard reported in 1998, based on unpublished personal observations, that carnitine deficiency was common in autism [12]. Filipek et al. in 2004 found low total and free carnitine in affected children [13]. In the "Study of Toddlers with Autism and Regression" (STAR), 7/21 subjects (ages 21–41 months, mean 28.4) with regression had a free carnitine value significantly below the normal range and another two were at the lower limits of normal; 4/13 with autism but without regression had values below the normal range (Alvin Loh, personal communication). In 2005, Mostafa et al. [14] noted dramatically low levels of carnitine and polyunsaturated fatty acids (PUFA) and elevated lactate levels in autistic children in Cairo between 4 and 12 years of age. In 2015, this group again reported strikingly low levels of carnitine and PUFA, including docosahexaenoic acid (DHA), this time in children from Saudi Arabia [15]; the findings were highly statistically significant in both studies compared to age- and sex-matched controls. The degree of laboratory abnormalities and the older age of the children suggest the possibility that an extremely deficient diet in an older age group might reveal biochemical findings that might be only transient on a different diet.

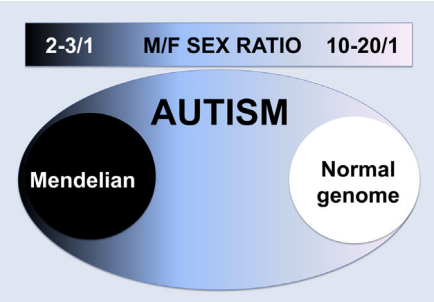

Figure 1. The sex ratio across the autism spectrum.

The importance of nutrition during the first 1,000 days (conception through age 2 years) is recognized as especially important for growth and development [16]. We do not detect evidence of in utero deficiency, and the content and bioavailability of carnitine in breast milk, infant formulas, and cow's milk likely provide protection in the first 3–6 months of life. We propose that NoMeND infants would typically be normal at birth and display normal early development but would begin manifesting pathology between 6 months and 3 years of age, with the greatest risk between 6 and 18 months concomitant with a typical fall in dietary carnitine intake as juices, fruits, cereals, and vegetables – but not meats – are introduced into the diet. A picky eater who dislikes meat (especially red meat) could easily have insufficient carnitine intake between 6 and 36 months of age; resistance to new foods is frequently reported in autism [17].

There is substantial evidence that maternal genotype and carnitine levels determine the carnitine status of infants at birth, with asymptomatic mothers with biallelic systemic primary carnitine deficiency leading to transient severe,

Table 1. Examples of very high male/female ratios in autism spectrum disorders

M/F ratio	Comments	References
23/1	Subset with normal physical exam and brain imaging	[7]
22/1 (6.8/1)	For Asperger (overall)	[103]
15.7/1	6 year follow up of 50 cases of childhood autism	[104]
12/1	Asperger	[105]
9/1	General	[106]
8/1	Autistic children in mainstream schools	[107]
5.6/1 to 6.1/1	Taiwan registry (increasing trend over time)	[108]

but apparently benign, carnitine deficiency in their heterozygous unaffected offspring [18, 19]. These heterozygous infants are often detected through newborn screening and are not known to go on to develop autism. Newborns with biallelic systemic carnitine deficiency are occasionally detected by newborn screening, but the reliability is unknown, and positive tests more often result from maternal than from infant deficiency [19].

Although low plasma carnitine is reported in autism, one might reasonably ask why it is not reported more often. There are at least three possible reasons: first, we suspect only 10–20% of autism is caused by brain carnitine deficiency; second, it is brain carnitine and not plasma carnitine that is critical, and brain deficiency might occur with a normal plasma level; and third, affected infants might have had low plasma carnitine during the crucial developmental period, at the time of symptom onset, but have their plasma carnitine return to normal when measured, typically, at 3–10 years of age.

Other micronutrient deficiencies have been reported in autism, including magnesium, zinc, selenium, and vitamins A, D, E, and B complex [20]. Brain deficiency of essential PUFAs, especially DHA, also deserves consideration, as noted above [21–23], and one review observed that "the majority of studies on attention-deficit/hyperactivity disorder (ADHD) and autism found a significant decrease in DHA levels" [24]. Whether brain deficiency of carnitine or PUFA might relate to the etiology of ADHD is beyond the scope of this discussion, but we note that ADHD also affects far more males than females. The intriguing possibility that carnitine deficiency might itself cause PUFA or DHA deficiency is suggested by the fact that DHA synthesis requires carnitine to move fatty acids between subcellular compartments, including the peroxisome [25]. There are multiple combinations regarding the possible roles of PUFA and carnitine deficiency n autism: i) either alone might cause autism; ii) combined deficiency might cause autism; and iii) carnitine deficiency might cause secondary PUFA deficiency and even might mediate most of its harm through this secondary deficiency.

Mechanisms of sex bias, carnitine deficiency, and the *SLC6A14* gene

Any serious student of autism is immediately aware that by far the single biggest genetic risk factor is male rather than female sex. The mechanism of the male sex bias in autism has been debated widely [26]. One contributing factor is clearly deleterious mutations in genes on the X chromosome (e.g. fragile X syndrome, *IL1RAPL1*, *NLGN4X*), but this does not account for the extreme sex bias particularly in the milder patients where such mutations usually are not found (Table 2). For sex bias associated with autosomal genes, the most common mechanism is that the susceptibility is mediated by maleness itself as determined by the SRY gene and the extensive secondary hormonal differences between males and females. Classically this would be referred to as sex-limited inheritance and is exemplified by the sex differences in pattern baldness in males and by female susceptibility to breast cancer with mutations in *BRCA1* or *BRCA2*. The hypothesis of the extreme male brain [27, 28] involving higher fetal levels of testosterone would fit a sex-limited model with the susceptibility mediated by maleness and the SRY gene. Similarly the effect of sex bias in CNVs suggesting that females are more resistant to developing autism than males with a given autosomal genetic load [6, 29, 30] could fit with a sex-limited model mediated by SRY and sex

Table 2. Mechanisms for high male/female ratio

Mechanism	Examples
Deleterious mutation in genes on the X chromosome	Fragile X, *IL1RAPL1*, *NLGN4X*
Sex-limited by SRY and hormonal effects	*BRCA1/2* mutations
	Male pattern baldness
	Autism caused by certain autosomal CNVs?
	Extreme male brain hypothesis?
Sex-limited by genes escaping X-inactivation	*SLC6A14* and autism?
	Autism caused by certain autosomal CNVs?
Sex limited by genomic imprinting with females having a paternal X while males do not	Relevant to Turner syndrome [109] but relevance to healthy XX females and XY males unknown
Y linked inheritance	Maleness
Unique X and Y inactivation	*VAMP7* (aka SYBL1) as a special case, random inactivation in females, Y inactive in males [110]

hormone differences. The proposal for a unified genetic theory for sporadic and inherited autism [29] likely represents mostly sex-limited inheritance mediated by SRY. Another possibility for generalized sex differences would be effects of genes that are on the X chromosome but not subject to X-inactivation. These genes would be expressed at higher levels in females compared to males, and might directly protect females or modify the sex ratio for a phenotype mediated by an autosomal genotype. Thus one can define at least two forms of sex ratio bias, those mediated by SRY and those mediated by X-linked genes escaping random X-inactivation. Another hypothetical mechanism for male/female bias would be through genomic imprinting on the X chromosome, since both males and females have a maternally derived X but only females have a paternal X (Table 2). Another possible mechanism for male bias would be Y-linked inheritance with de novo mutations or incomplete penetrance with inherited mutations, but the lack of evidence for de novo mutations on the Y chromosome and minimal father to son transmission do not support this possibility. The male sex bias in NoMeND autism should be explained by one of these mechanisms. Our hypothesis might predict that such a mechanism would involve a gene involved in carnitine synthesis or transport.

There happens to be one gene, *SLC6A14*, which is involved in carnitine transport across the BBB, and it is almost certainly not subject to transcriptional X-inactivation in mouse or human, possibly making it the lynchpin of the extreme male bias. The lack of X-inactivation represents a mixed epigenetic and genetic regulatory mechanism. There is no CpG island associated with the promoter and no differential methylation of the active and inactive X as occurs for genes subject to random X-inactivation (Genome Browsers and manuscript in preparation, Jimenez-Rondan and Beaudet). The gene is expressed in colonic epithelium (mouse but not human), epidermis of skin, airway epithelial cells, retina, and cultured brain endothelial cells considered to be a model for BBB transport [31]. Its effect could be viewed as a male susceptibility or as a female protection for brain carnitine deficiency.

The product of the *SLC6A14/Slc6a14* human/mouse gene is an amino acid transporter designated as $ATB^{0,+}$. The SLC6A14 protein transports not only carnitine but also 18 of the 20 amino acids found in proteins [32]. The gene was cloned in 1997 [33], and in 2001, this protein was reported to transport carnitine [34]. The SLC6A14 protein was reported to play a role in the blood-brain barrier transport of carnitine [35] including in a cultured cell line regarded as a model for blood bran barrier transport [36]. There is evidence that both the SLC6A14 protein and the OCTN2 transporter encoded by *SLC22A5* function at the BBB [36]. The *SLC6A14* gene is very highly expressed in estrogen receptor positive but not estrogen receptor negative breast cancer cell lines [32]. The extent to which estrogen induces expression of *SLC6A14* and possibly BBB transport in mouse or human in vivo is unknown, but could be studied in the mouse.

How do the nutritional aspects of carnitine relate to the hypothesis?

Carnitine is extremely abundant in red meat; is very scarce in fruits, cereals, and vegetables; and has intermediate abundance in other animal foods including chicken, fish, eggs, and milk. One gram of beef contains 2000 times more carnitine than a gram of white rice. The major role of carnitine is to enable the transfer of fatty acids into mitochondria, and its distribution roughly parallels that of mitochondria. Carnitine facilitates entry of fatty acids into mitochondria by forming covalent acyl-carnitines (Fig. 2A). Carnitine is abundant in human and rodent cardiac and skeletal muscle, liver, kidney, and brain [37]. It is widely stated that meat eating humans derive about 75% of their carnitine from the diet and 25% from endogenous synthesis, although it is difficult to find primary data to support this statement, and a much smaller fraction would likely be derived from a strict vegetarian diet [38]. Dietary carnitine is a mixture of free carnitine, acetyl-carnitine, and lesser amounts of other acyl-carnitines. The potential for biosynthesis of carnitine by the microbiome is uncertain. Carnitine can be synthesized by yeast such as Candida species [39], but not by bacteria as far as known at present [40]. Dietary carnitine can be metabolized by bacteria in the gut [40].

For the carnitine deficiency hypothesis, the sources of carnitine in the brain are of greatest interest. The concentration of free carnitine in plasma ranges from 25 to 60 nmol/liter, while the concentration in spinal fluid is about 25-fold lower at 0.7–2 nmol/liter [41]. This suggests that carnitine is not well transported across the BBB. Thus it may be that a much larger fraction of carnitine in brain is derived from endogenous synthesis than is the case for carnitine in somatic tissues. On the other hand, most males with *TMLHE* deficiency develop as healthy adults, so the brain can acquire sufficient carnitine for normal health in most cases even when endogenous synthetic capacity likely is extremely limited.

How is carnitine synthesized in humans?

Carnitine can be synthesized by most or all mammals [42, 43]. Its synthesis starts from trimethyllysine that is derived from protein degradation (Fig. 2B). *TMLHE* encodes TML dioxygenase which converts TML to hydroxyl-TML (HTML) as the first step in the pathway. The next two steps convert HTML to γ-butyrobetaine (also known as deoxycarnitine). *BBOX1* converts γ-butyrobetaine to carnitine as the last step in the pathway. Although some publications state that carnitine is not synthesized in the brain [44], there is strong evidence that the brain can synthesize carnitine [42].

How does knowledge of primary systemic carnitine deficiency relate to the hypothesis?

Primary systemic carnitine deficiency (PSCD, OMIM 212140) is a human disorder caused by biallelic loss-of-function mutations in *SLC22A5* [45]. Deficiency results in failure to

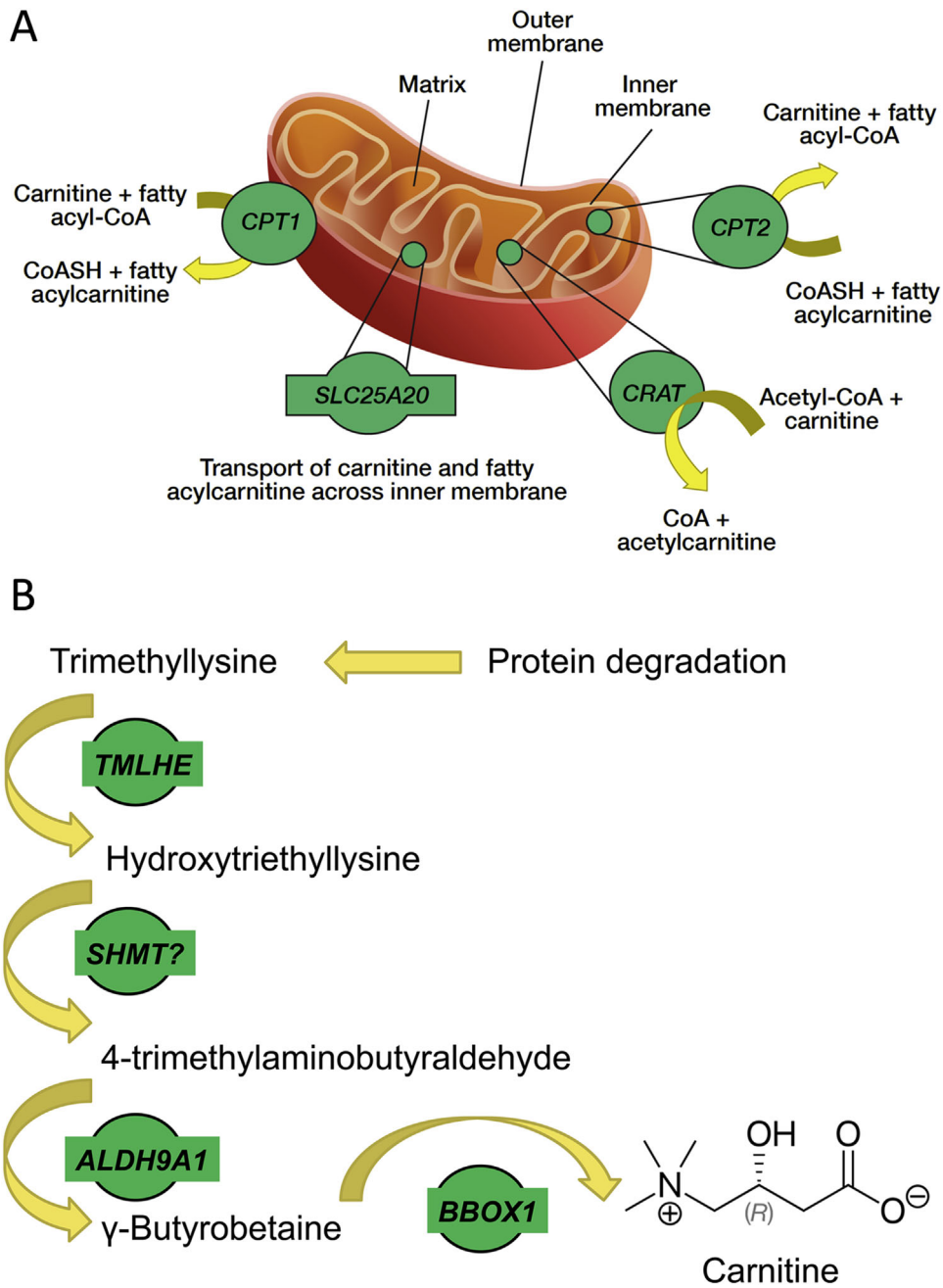

Figure 2. Biosynthesis and metabolism of carnitine. **A**: Role of carnitine in mitochondria. Abbreviations/gene symbols: *CPT1*, carnitine palmitoyltransferase I; *CPT2*, carnitine palmitoyltransferase II; *CRAT*, carnitine acyltransferase; *SLC25A20*, gene symbol for carnitine-acylcarnitine translocase (CACT). **B**: Carnitine biosynthesis from trimethyllysine (TML). Abbreviations/gene symbols: *ALDH9A1*, aldehyde dehydrogenase, family member 9A1; *BBOX1*, gamma butyrobetaine hydroxylase; *SHMT*?, serine hydroxymethyltransferase, unspecified; *TMLHE*, N-trimethyllysine hydroxylase, epsilon.

adequately resorb carnitine in the kidney causing substantial urinary losses. The encoded protein, OCTN2, imports carnitine into the renal tubular and other cells, and it likely plays a role in gastrointestinal and placental transport and in transport across the BBB. Patients with PSCD often develop severe carnitine deficiency and may suffer life-threatening metabolic decompensation. Later onset of symptoms may include cardiomyopathy, and some individuals may remain asymptomatic [46]. There is evidence that heterozygous deficiency for *SLC22A5* is associated with slightly lower plasma carnitine levels in healthy adults [47].

One might think that autism would occur with increased frequency in PSCD, but this is rarely if ever reported, although there is one report of developmental delay [48]. The median age at diagnosis for infants presenting with hypoglycemia or muscle weakness is 1.5 years, so some infants may be placed on carnitine supplementation early enough to prevent brain carnitine deficiency. Likely more important is the fact that the brain can synthesize carnitine so long as the infant is not *TMLHE* deficient, and the carnitine deficiency in PSCD likely affects somatic tissues more prominently than brain. We hypothesize that *SLC22A5* heterozygous deficiency might be a risk factor for autism.

Reports from the Faroe Islands are intriguing in this regard. There is an incidence of 1 in 300 for homozygous *SLC22A5* deficiency in this population isolate with many individuals being asymptomatic or presenting as adults with cardiomyopathy or cardiac arrhythmia [46]. This would imply a carrier frequency of about 1 in 35. Interestingly, autism is relatively frequent in the Faroe Islands with a reported rate 0.56% in 2002 and 0.94% in 2009 [49]. The Faroese diet is rich in lamb (very high carnitine content) and fish. Remarkably 21 of 43 (49%) autism cases in 2002 and 16 of 24 (67%) in 2009 were said to have Asperger syndrome, which we interpret to mean that many or most of these patients were milder and would meet criteria for NoMeND autism. There is no precedent for such a high proportion of Asperger diagnosis in a general series of autism patients. We speculate that NoMeND autism caused by brain carnitine deficiency is especially common in the Faroe Islands, and that heterozygous or homozygous deficiency for *SLC22A5* might be a risk factor for NoMeND autism.

Could genes causing secondary carnitine deficiency be a risk factor for NoMeND autism?

Secondary carnitine deficiency is often seen in patients with inborn errors of organic acid or fatty acid metabolism (Supporting Information Table S1); see review [50]. Many different disorders can lead to secondary carnitine deficiency by causing accumulation of excessive levels of various acylcarnitines which are then lost through the urine. We hypothesize that heterozygous deficiency or unrecognized homozygous deficiency for a disorder causing secondary carnitine deficiency can be a risk factor for autism. Short-chain acyl-CoA dehydrogenase deficiency is caused by mutations in *ACADS* and one partially inactivating SNP in the gene is particularly common. Although six SNPs in *ACADS* are associated with increased levels of plasma butyrlcarnitine [51], the great majority of deficient patients detected by newborn screening experience a benign course [52]. We suggest that heterozygous or homozygous genotypes for partial or complete *ACADS* deficiency could be a common risk factor or modifier for NoMeND autism.

Could other carnitine-related genes act as risk factors?

Additional genes involved in the metabolism of carnitine can be divided into transporters and enzymes (Fig. 2A and Supporting Information Table S1). *SLC16A9* is a transporter which functions as a renal transporter but exports carnitine from the renal tubular cell to the plasma compartment. Human *SLC16A9* deficiency is not reported, but numerous single nucleotide polymorphisms (SNPs) including rs7094971 are associated with variation in plasma free carnitine levels [53]. A genome-wide association study (GWAS) examining plasma metabolites in healthy adults identified 20 different genes with SNPs associated with altered carnitine or acylcarnitine levels in plasma [54] (Supporting Information Table S2) (http://metabolomics.helmholtz-muenchen.de/gwa/si/).

When do symptoms start and do they include regression?

We hypothesize that onset of symptoms for NoMeND might occur after starting non-meat solid foods and juices, and dietary intake of carnitine when starting solid food may be a critical variable. Minor illnesses sometimes associated with hospitalization, especially gastrointestinal, might decrease oral intake of carnitine and increase urinary losses via renal tubular dysfunction. Chronic gastrointestinal symptoms are common in autism [55] and might impair intake and intestinal absorption of carnitine. Onset of symptoms in autism often occurs in the 6–18 month age range. If an infant has a social smile and is very interactive at 5–7 months of age, but loses the social interactions by 12–18 months of age, this may or may not be recognized as a subtle form of regression.

Has the prevalence of autism changed?

There is no doubt that prevalence estimates of autism have increased over the last two decades, but there is expert opinion that this is due at least in part to broadening of the diagnostic criteria, diagnostic switching from entities such as intellectual disability, and increased awareness of autism among parents and medical professionals [56]. The Centers for Disease Control and Prevention (CDC) has published a series of reports on the prevalence of autism. Although there may or may not be a true change in the prevalence, the report in 2007 noted as follows: "Over the last decade, the most notable change in characteristics of children identified with ASD through the Autism and Developmental Disabilities Monitoring Network is the growing number who have average or above average intellectual ability. This proportion has increased consistently over time from 32% in 2002, to 38% in 2006, to 46% in 2010, or almost half of children identified with ASD" [57]. The suggestion of a trend to higher IQ and the many environmental variables hypothesized for brain carnitine deficiency could be compatible with a true increased prevalence of NoMeND autism, but data are not adequate to make such a conclusion. In addition, the overall intake of beef by the US population has dropped significantly since the 1970s, and this could potentially increase the prevalence of NoMeND autism.

Mitochondrial abnormalities generally in autism

There are numerous reports of specific and nonspecific abnormalities of mitochondrial function in autism, and a review and meta-analysis in 2012 provides an extensive bibliography [58]. Highly penetrant mutations in nuclear encoded mitochondrial genes or in the mitochondrial genome are not a frequent cause of autism. To the extent that there are nonspecific mitochondrial abnormalities in autism, deficiency of carnitine and/or PUFAs could underlie these findings. The 2012 review states that "Carnitine was the most commonly noted supplement to be helpful." There are also many reports of oxidative stress or abnormalities of redox metabolism [59]. Again these findings might be secondary to carnitine or PUFA deficiencies.

There are other mitochondria-related hypotheses being studied that might or might not be compatible with the hypothesis proposed here. A search of PubMed identifies 150 citations linking mitochondria and autism as of March 2017, 158 linking glutathione and autism, 51 linking docosahexanoic and autism, and 47 linking propionic and autism. The evidence that PUFA/docosahexanoic acid might relate to this hypothesis was mentioned above. The suggested involvement of propionic acid may fit well with the carnitine deficiency hypothesis. Increased propionic acid would likely lead to carnitine losses through excretion of propionyl carnitine. The groups of MacFabe and Frye have published extensively on the potential importance

of propionic acid including its use as a food preservative [60–62]. There is evidence that increased propionate could result from changes in the microbiome which in turn relates to gastrointestinal disorders and mitochondrial function [61, 63, 64]. At least four reviews of the relevance of the microbiome to autism appeared in 2016 [65–68], and the potential for interaction with brain carnitine deficiency could be substantial. The potential to manipulate the enteric microbiome therapeutically is being explored [69]. Dietary carnitine can be metabolized by bacteria in the gut [40]. Bacterial conversion of carnitine to trimethylamine-N-oxide (TMAO) in the gut has received attention as a risk factor for cardiovascular disease with high starch intake possibly a factor [70–73].

What might be the role of drugs?

Many drugs could potentially contribute to carnitine deficiency. Antibiotics (especially penicillins, cephalosporins, and clindamycin) have the ability to engender major changes in normal bowel flora [74]. Some β-lactamase resistant antibiotics including ampicillin can inhibit the OCTN2 carnitine transporter encoded by *SLC22A5* in vitro, but in vivo effects are not clear [75]. Antibiotics conjugated with pivalic acid to increase absorption are especially dangerous apart from their antibiotic action because pivalic acid forms pivaloylcarnitine, which is excreted by the kidney depleting carnitine [76–78]. Pivalic acid is also found in nipple-fissure unguent and can cause metabolic changes in breast feeding infants [79].

Valproic acid is well known to deplete carnitine [80], and its use is often accompanied by carnitine supplementation. Omeprazole, sold under the brand names Prilosec and Losec, is a proton pump inhibitor often prescribed in children with gastroesophageal reflux disease [81]. Omeprazole is a potent inhibitor of the OTCN2 carnitine transporter, although its ability to cause carnitine deficiency in vivo is speculative [82]. Interestingly, mutations in two proton pump genes (*SLC9A6* and *SLC9A9*) cause intellectual disability with autism features [83], but there is no evidence that carnitine deficiency is part of the pathogenesis. Meldonium is a limited market drug more available in eastern Europe, but easily obtainable on the internet [84]. It is marketed as an antiischemic drug and also may have anticonvulsant and antihypnotic effects. It is banned for use by athletes. It is an analogue of γ-butyrobetaine and inhibits the BBOX1 carnitine biosynthesis enzyme and the OCTN2 transporter. It has a potent ability to produce carnitine deficiency in rodent models [85] and could be very dangerous if given to infants.

Carnitine as a conditionally essential nutrient

Carnitine was initially designated vitamin B_T, but more recently it has been judged not to qualify as a vitamin. At present, the US National Institutes of Health Office of Dietary Supplements (https://ods.od.nih.gov/factsheets/Carnitine-HealthProfessional/#h2) states as follows:

> "Healthy children and adults do not need to consume carnitine from food or supplements, as the liver and kidneys produce sufficient amounts from the amino acids lysine and methionine (via trimethyllysine) to meet daily needs (1-3; see website for references). The Food and Nutrition Board (FNB) of the National Academies reviewed studies on the functions of carnitine in 1989 and concluded it was not an essential nutrient. The FNB has not established Dietary Reference Intakes (DRIs) – including a recommended dietary allowance (RDA) – for carnitine."

We question this recommendation. Synthesis of carnitine is clearly limited in 1 in 350 males who have *TMLHE* deficiency. In addition, it is known since at least 1981 that otherwise healthy infants given unsupplemented soy based formulas can develop symptomatic carnitine deficiency [86, 87].

The potential essentiality of carnitine as a nutrient has been known for decades [88]. In 1987, Rebouche stated "Until this (additional) information is available, it is inappropriate to include or exclude carnitine as an essential nutrient for the human infant [89]." We would agree with this statement today. In 2004, an NIH sponsored symposium was held entitled "Carnitine: The Science Behind a Conditionally Essential Nutrient." <https://ods.od.nih.gov/News/Carnitine_Conference_Summary.aspx>. The report from this meeting restates the Office of Dietary Supplements opinion above with little new data. None of the available data effectively address whether a very low carnitine diet is adequate for normal or optimal development and whether adequate carnitine is present in the brain given the effect of the BBB which mediates ~25-fold difference in concentration between higher plasma and lower CSF levels.

Potential value of meat in early feeding of solid foods

A series of studies carried out under the auspices of The Nutrition Collaborative Research Support Program (NCRSP) reported better growth and cognitive function in children in Egypt, Kenya, and Mexico receiving supplements from animal source foods; see bibliography in Neumann et al. [90]. One study in Kenya found that children fed meat supplements over 2 years had significant improvements in test scores for arithmetic, languages, and geography compared to children receiving non-animal or no supplements [90]. Some benefits have been reported using meat as an early complementary food in breastfed infants both in China [91] and in the USA [92]. Thus perhaps it is not unreasonable to worry that unrecognized brain deficiency of carnitine in infancy might cause suboptimal cognitive development and perhaps abnormalities of learning and behavior including autism.

Does breast feeding protect against autism?

The possibility that breastfeeding offers a protective effect against autism seems to have attracted little recent attention despite some intriguing reports. A study in 1989 from Japan [93] found that 24.8% of the autism patients and 7.5% of the controls ($p < 0.005$) were weaned by the end of 1 week, and the authors concluded that early weaning may contribute to the etiology of autism. Another publication from Oman in 2012 [94] suggested benefits from increasing periods of exclusive breastfeeding and from continued breastfeeding. In contrast, a very recent study failed to detect any relationship between breastfeeding and autism [95]. Breastfeeding may be protective in some circumstances and not others depending on the genotype of the infant and the carnitine content of the alternative calories which vary widely between commercial infant formulas, cow's milk, and a mix of fruits, cereals, vegetables, and meats.

Testing the hypotheses

There are multiple ways that this hypothesis might be tested (Table 3), and we have begun such efforts starting with mouse studies. Mice with a null mutation in *Slc6a14* were reported to be resistant to spontaneous breast cancer [96], and a second null allele is available through the Knockout Mouse Project <https://www.komp.org/>. Phenotypic analysis through the International Mouse Phenotyping Consortium <http://www.mousephenotype.org/> did not detect major abnormalities. We have initiated studies to compare transfer of radioactive carnitine across the BBB in control male mice compared to control female mice and in comparison to male and female *Slc6a14* null mice (manuscript in preparation, Jimenez-Rondan and Beaudet). Comparison of control male and female primates is also feasible. Noninvasive in vivo methods for determining brain carnitine levels in children would be extremely valuable.

There are now numerous cohorts of autism families and controls from both simplex and multiplex families. In many cases, SNP genotyping data are available as well as whole exome sequencing and limited whole genome sequencing data. It is feasible to perform GWAS and candidate gene (see Supporting Information Tables S1 and S2) studies to assess whether genetic variations may act as modifiers for risk of NoMeND autism. Modifier genotypes could be sought comparing *TMLHE* deficient autism probands and *TMLHE* deficient healthy males and comparing NoMeND probands to non-NoMeND probands in large collections.

We propose that the published work of the Baby Siblings Research Consortium (BSRC) provides an ideal background for testing the carnitine/micronutrient deficiency hypotheses. The BSRC has reported the recurrence risk for newborn siblings born to families with one or more children already diagnosed with autism (Fig. 3) [97]. In a related publication it appears that the cohort being studied represents the milder end of the autism spectrum (82) and thus be greatly enriched for cases of NoMeND autism. The Baby Siblings investigators found a frequency of 29.5% for autism in male siblings and 9.8% in female siblings. A risk of ~45% was observed if the new infant was male and there was more than one previous child with autism, suggesting to us the possibility of one very strong additional modifier. These recurrence risks are far above the usually reported for autism in the early 2000s of 2–8% [98, 99], although there are more recent reports of higher recurrence risk especially in males born to families with two previous affected males [100, 101]. Recurrence risk is obviously extremely heterogeneous even when the etiology is known to be Mendelian being very low in cases of de novo CNVs or point mutations compared to quite high for inherited dominant mutations or instances of autosomal recessive or X-linked inheritance. The Baby Siblings data suggest to us that the recurrence risk for NoMeND autism might be relatively high with a few key modifiers, although environment and especially diet would be critically important.

A recent publication of the IBIS (Infant Brain Imaging Study) Network [102] is remarkably consistent with NoMeND autism with striking parallels. They report "that hyper-expansion of the cortical surface area between 6 and 12 months of age precedes brain volume overgrowth observed between 12 and 24 months in 15 high-risk infants who were diagnosed with autism at 24 months." This is consistent with what we hypothesize for NoMeND autism. They conclude that "that early brain changes occur during the period in which autistic behaviors are first emerging." This study had very extensive exclusion criteria as described in their methods so that the sample would likely be highly enriched for what we term NoMeND autism. We hypothesize that brain carnitine deficiency can cause brain overgrowth.

A therapeutic trial could enroll families in a manner as analogous as possible to the description of the BSRC [97], and explore the effects of various dietary interventions. With an overall recurrence risk of 17.8%, a modest sized cohort could detect a major reduction in recurrence risk.

Table 3. Opportunities for testing the brain carnitine deficiency hypothesis

Test whether female rodents and primates transport radioactive carnitine across the blood-brain barrier better than male counterparts.
Test whether transport of carnitine across the BBB is impaired in Slc6a14 null mice.
Possibly study transport across the BBB in humans using stable isotopes.
Attempt to develop methods to quantitate brain carnitine using in vivo imaging methods.
Perform GWAS and analyze genetic variations in candidate genes to identify modifiers for autism risk in NoMeND autism compared to other autism or and controls and test in TMLHE deficient autism males compared to TMLHE deficient healthy males.
Test whether dietary supplementation with carnitine, PUFA, or other micronutrients in infancy can reduce the risk of autism in siblings of NoMeND autism probands.

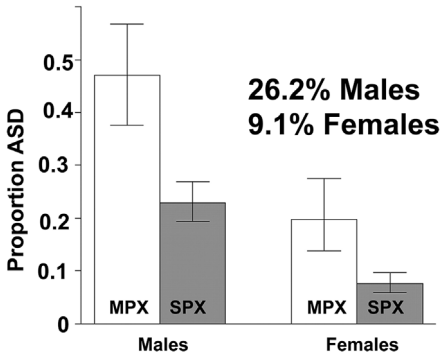

Figure 3. Recurrence risk for autism in Baby Siblings Research Consortium. MPX = multiplex, more than one previous child with autism. SPX = simplex, only one previous child with autism. With permission [97].

Interventions could include supplementation with individual metabolites such as carnitine, supplementation with various combinations of metabolites (carnitine and PUFA), and supplementation with a natural food such as beef or meat as the first complementary food. The problem of a blinded placebo control group is challenging because parents will desire the maximum chance to prevent autism in the new infants. To reduce this concern, participants could be monitored for plasma carnitine and PUFA levels, and withdrawn from the study if levels fell below a cutoff value. While supplementing with beef as a first complementary food is simple and low in cost, one probably would not want to encourage a preference for beef or meat that would persist into adult life because of the high fat content and reports that production of TMAO with high carnitine intake can increase risk of coronary artery and other disorders. Supplementing with carnitine fortification would avoid concern about promoting beef intake in adults and might be more acceptable to vegetarian families. Historical controls from the previous BSRC publications would already be available. If one were to use natural food products, meat and cereal could be compared as has been done in one recent trial in the USA [92]. In this case starting the solid foods at the earliest possible age would be desirable, although the American Academy of Pediatrics and the World Health Organization recommend against starting supplemental foods before 6 months of age. Additional variations of control groups could be envisioned.

Conclusions

We propose that there is suggestive evidence that brain deficiency of carnitine and perhaps other micronutrients including PUFA may cause NoMeND autism. Early treatment with carnitine and other micronutrient supplementation may benefit recently symptomatic children and may prevent recurrence risk both in families and in the general population risk. We suggest that a prevention trial should be carried out in families with infant siblings. The hypothesis can be tested in animal models to assess transport of carnitine across the BBB and to test for any phenotypic abnormalities resulting from brain carnitine deficiency. A method should be sought to measure brain carnitine noninvasively in vivo in children using imaging methods. The potential need for a minimum daily requirement for carnitine intake in infants should be reexamined.

Acknowledgements

Robin P. Goin-Kochel, Christian P. Schaaf, Dianne X. Dang (all Houston) and Frederic Vaz (Amsterdam) have made extraordinary contributions over 4 years to endless discussions and to attempts to gather information regarding the hypothesis. Laboratory colleagues including Jun Ge, Hongmei Xu, Dake Zhang, and Felix Jimenez-Rodan have conducted many preliminary studies attempting to test the carnitine hypothesis, some of which may never be published. Thanks to Alvin Loh (Surrey Place, Toronto) for permitting a personal communication. Editing help was generously provided by Vicky Brandt. The evolution of this hypothesis was supported by past grants from the Simons Foundation Autism Research Initiative (SFARI), Autism Speaks #7697, and currently the NIH Baylor College of Medicine Intellectual and Developmental Disability Research Center grant P30 HD024064.

At the time of the discovery of *TMLHE* deficiency, a patent (US20130005806 A1) was filed, but Baylor College of Medicine has since abandoned the pursuit of this patent. The author is a Professor at Baylor College of Medicine (BCM) and advisor to Baylor Genetics (BG). BG is a for-profit joint venture partially owned by BCM and majority owned by Miraca Holdings of Japan. BG offers commercial laboratory testing for a wide range of genetic disorders including for autism.

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