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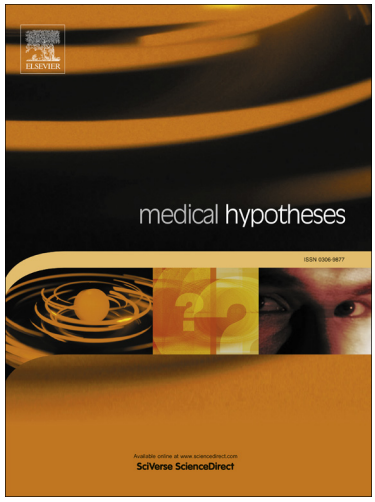

Autism: Will vitamin D supplementation during pregnancy and early childhood reduce the recurrence rate of autism in newborn siblings?

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TITLE PAGE

Autism: Will vitamin D supplementation during pregnancy and early childhood reduce the recurrence rate of autism in newborn siblings?

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ABSTRACT

Background

Vitamin D deficiency is widespread in the world including the vulnerable group of pregnant women. Vitamin D deficiency during pregnancy is hypothesized to contribute to the cause of autism. Further, it is hypothesized that vitamin D supplementation during pregnancy and early childhood will reduce the recurrence rate of autism in newborn siblings.

Methods

To investigate the hypothesis an open label prospective study was performed prescribing vitamin D during pregnancy to mothers of children with autism at a dose of 5000 IU/day. The newborn siblings were at high risk for the recurrence of autism. The newborn infants were also prescribed vitamin D, 1000 IU/day to their third birthday. The newborn siblings were followed for three years and during that time, were assessed for autism on two separate occasions: at 18 months and 36 months of age. The results were compared to the reported recurrence rates in siblings of autistic children in the literature.

Results

The final outcome was 1 out of 19 (5%) developed autism in contrast to the recurrence rate of approximately 20% in the literature. We did not have a control group, nor was there blinding.

Conclusions

The results are promising, however, this is a preliminary study with very small numbers and was uncontrolled. Further study with larger numbers is indicated. The ethics of prescribing a low dosage of vitamin D such as 400 IU D3/day to a control group of

mothers in comparison to a large dose such as 5000 IU D3/day are problematic in our opinion.

BACKGROUND

Autism (Autism Spectrum Disorder) is defined by social, communication and behavioral symptoms (1). There is no consensus about its causation. Interactions between genetics and an unknown environmental factor are both thought to contribute to its cause(s). There is a debate as to whether the genetic risk of common variants is as large as 50% (2) or small as 1% (3). De novo mutations are reported at 2.6% (2). Autism has increased to the current rate of approximately 1% from a rate of 0.05% in the 1970s.

Cannell has hypothesized that vitamin D deficiency during pregnancy may contribute to autism (4). Vitamin D deficiency is reported to be widespread worldwide (5). There is still a debate about what the normal levels of vitamin D should be; whether it should be equal to, or more than, 20 ng/ml, 30 ng/ml or higher even still (6).

There have been numerous studies showing vitamin D levels in children with autism are lower than normal (See Pioggia et al for a review) (7). However, it is unclear if children with ASD are born with lower vitamin D levels, as Cannell hypothesized, or develop low levels due to less sun exposure than typically developing children. If children with ASD are born with genetically lower vitamin D levels, then vitamin D may be the genetic/environmental factor long sought in ASD.

Three recent studies point to the possibility that low vitamin D levels in children with ASD are genetic. First, Kočovska´ et al found those ASD had significantly lower vitamin D levels than their siblings in an environment with little sunshine, raising the question if the differences were genetic (8). Second, Fernell et al identified 58 Swedish sibling pairs, one with ASD and one without, and found vitamin D levels at birth were lower in those with ASD (9). Third, Schmidt et al examined associations between ASD and

common, functional polymorphisms in vitamin D pathways in the CHARGE cohort and found polymorphisms that lower vitamin D levels were much more common in ASD children (10). These studies support the hypothesis that children with ASD have genetically lower vitamin D levels.

Cannell also hypothesized that large vitamin D doses would have a treatment effect in autism (11). Two recent papers have supported that theory. First, Saad et al found that 5,000 IU/day of vitamin D3 given to 86 ASD children (aged 3-9 years) for 3 months showed 80% of them had significant improvement on the CARS (12). Also a recent case report in Pediatrics found that a 36-month-old toddler with ASD and rickets showed dramatic improvement in three different standard ASD rating scales after being given 150,000 IU/month of vitamin D3 for two months (13).

However, randomized controlled trials of high dose vitamin D3 in children with ASD have not been conducted.

Cannell and Grant discuss mechanisms by which vitamin D may contribute to autism (14). First, they describe how vitamin D contributes to DNA repair genes. Vitamin D deficiency could conceivably contribute to the many de novo gene mutations reported to contribute to autism because those mutated genes have not been repaired (15). Reactive oxygen species (ROS) are a part of the normal metabolic function of cells of the body and because the oxygen is unstable, ROS also contributes to DNA damage on a regular basis. Thus, the human genome needs to constantly repair itself. If the genome did not repair itself, there would be a much higher incidence of cancer and other major defects.

Second, Cannell and Grant report that vitamin D plays a major role in the immune system. Evidence exists of neuroglial activation and neuroinflammation in the brain of

patients with autism (16). Vitamin D is important in up-regulating production of antioxidants including glutathione, superoxide dismutase, and thioredoxin reductase (17). Thus, by genetically up-regulating antioxidants, vitamin D could reduce neuroglial activation and neuroinflammation.

Third, Van de Water et al. have reported autoimmune conditions in autism including the presence of maternal antibodies to fetal brain tissue (18). Mostafa et al also found brain autoantibodies in 50 children with ASD (19). They also found vitamin D blood levels were inversely and strongly associated with the absolute blood level of those antibodies ($R = -0.86$, $p<0.001$). Vitamin D has a major role in inducing T regulatory cells which have an effect on controlling antibodies contributing to autoimmune conditions (20). Thus, the vitamin D induced T regulatory cells may have a role in reducing autoimmune conditions and protecting the fetus.

In addition to Cannell and Grant's discussion of possible vitamin D factors contributing to autism, autism has been described as having synaptic pruning defects during brain development (21). Pruning likely takes place through macroautophagy via the AMPK/mTOR pathway (adenosine monophosphate kinase/mammalian target of the rapamycin signaling pathway) (22). The mTOR pathway is thought to be involved in autism (23,24). Vitamin D plays a major role in autophagy through its effect on AMPK/mTOR pathway (25). Microglia are also thought to play a role in pruning of synapses during development of the brain (26,27). Serotonin modulates developmental microglia during synaptic refinement (28). Patrick and Ames have reported that vitamin D activates the transcription of the serotonin synthesizing gene tryptophan hydroxylase 2 (TPH2) in the brain at the vitamin D response element (VDRE) and represses the transcription of tryptophan hydroxylase 1 (TPH1) in tissues outside the blood-brain barrier (29,30). According to them, this explains the "serotonin paradox" which is low

concentrations of serotonin in the brain of children with autism but elevated concentrations of serotonin outside the blood brain barrier. Serotonin is an important brain neurotransmitter and affects the development of the brain (31).

Vitamin D is important for the developing brain during pregnancy (32). Bodnar et al. report a high prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates (33). Vitamin D deficiency during pregnancy has been reported to be associated with pre-eclampsia, pre-term birth, small-for-dates, and gestational diabetes (34,35,36). Furthermore, these conditions of pregnancy have been reported to confer a risk for autism (37,38). In addition to the above, vitamin D deficiency in pregnant women impairs regulatory T cell function (39). Vitamin D supplementation during pregnancy prevents vitamin D deficiency in the newborn (40).

Vitamin D supplementation during pregnancy and its correlation with feto-maternal outcome has been reported by a number of investigators. Sablock et al. (2015) found that vitamin D supplementation reduces the risk of maternal comorbidities and helps improve neonatal outcomes (41). Bodnar et al. report that maternal vitamin D deficiency increases the risk of preeclampsia (42). Wagner et al. report that vitamin D status during pregnancy demonstrates lower risk of preterm birth with higher vitamin D closer to delivery (43). In contrast, Tabatabaei et al., report that dietary vitamin D intake during pregnancy in guinea pigs does not affect the already high rate of gestational diabetes mellitus, whereas, higher doses of vitamin D before pregnancy appears to be protective (44). Schmidt et al. found a lower risk of autism if mothers took folic acid (prenatal vitamins) during the periconceptual period in contrast to starting the folic acid after finding out they were pregnant (45).

We present a hypothesis to determine whether adequate supplementation of vitamin D to pregnant women and/or supplementation to their infants and toddlers will reduce the recurrence rate of autism in newborn siblings. Fernell et al., recently recommended that a larger cohort be followed prospectively to study whether or not adequate supplementation of vitamin D to pregnant women might lower the risk for ASD in the offspring (9). We present a hypothesis and we report the results of a prospective trial of vitamin D during pregnancy and to their infants and toddlers which reduced the recurrence rates in newborn autism siblings.

METHODS:

This is a prospective study using vitamin D during pregnancy of mothers of children with autism and following the newborn siblings until their third birthday to see if they developed autism or not. The mothers and their infants and toddlers were prescribed vitamin D to see if the recurrence rate of autism in the newborn siblings could be reduced. This project was approved by the Center for Autism and Related Disorders Inc. (CARD, Inc.), a federally approved Institutional Review Board (IRB). The mothers all signed a consent form. We recruited mothers who had one or more children who had been diagnosed with autism by a physician or psychologist and who were pregnant in the first, second or early third trimester. We recruited participants by placing several ads in the local newspaper, one national ad online through the Vitamin D Council and one ad through the Autism Research Institute. We also recruited from local doctors.

We measured 25 hydroxy D3 levels (25 OHD) and serum calcium levels before giving the mothers vitamin D. We repeated these measures in 2 months. Six women were taking vitamin D before getting pregnant. The rest were pregnant when starting vitamin D. Only two were in the deficient range of less than 20 ng/ml of 25 OHD when starting vitamin D. Of the ones starting vitamin D after getting pregnant, all but two were in the second trimester of pregnancy when starting the vitamin D. The other two were early in the third trimester of pregnancy. We prescribed 5000 IU D3/day during the pregnancy and 7000 IU D3/day while breastfeeding. If the mother was not breastfeeding, we prescribed 1000 IU D3/day for the baby up to the age of three. These doses were chosen on the recommendation of Dr. Cannell. The 25 hydroxy D3 and serum calcium levels were measured at either Quest labs, PAML (Pathology Associates Medical Laboratories) or a lab in Israel all using liquid chromatography/mass spectrometry (LC-MS/MS) methodology. The choice of the lab depended upon the proximity to where the

mother lived. Mothers were from the local area, from neighboring states, from across the United States, from Canada, and from Israel.

We monitored the outcome of the birth including birthweight, date of delivery as compared to expected date of confinement, method of delivery, and any complications.

At 18 months of age, we used a screening test for autism, the Modified Checklist for Autism in Toddlers (MCHAT). At 3 years of age, we used a questionnaire for diagnosing autism, called the Pervasive Developmental Disorder Behavior Inventory (PDDBI).

RESULTS:

See Table 1. Results of 25 OHD, MCHAT, and PDDBI

We had 17 of 19 (89%) pass the MCHAT screening test at 18 months of age, that is, they were negative for autism. We had 17 of 19 (89%) pass the PDDBI diagnostic questionnaire at 3 years of age. Eighteen of 19 (95%) had a final diagnosis as not having autism.

One child who did not pass the PDDBI developed an illness which included bilateral ear infections. After being treated with antibiotics, the child subsequently developed gastrointestinal difficulties. The child had passed the MCHAT at 18 months of age, but after the illness, regressed and eventually met the criteria for autism at 3 years of age. The child was treated for her GI problems, but continued to have difficulty with recurrent GI symptoms. The symptoms of autism seemed to coincide with the severity of GI problems. As the GI symptoms got more severe, the symptoms of autism got more severe. As the GI symptoms improved, so did the autism symptoms. There were times when the child apparently responded to metronidazole which is often used to treat Clostridium difficile. C. diff. becomes problematic after the individual is treated with an antibiotic like ciprofloxin or azithromycin for reasons such as ear infections, sinusitis, diverticulitis.

One other child who no longer meets the criteria for autism temporarily met criteria for autism. After getting ill and being treated with antibiotics, the child regressed and developed symptoms of autism. The child had Klebsiella cultured in his stool. On checking with the parents about both of the older siblings of these two children, we

discovered they also had illnesses before regressing to autism. Both also had GI problems after being treated with antibiotics.

DISCUSSION:

The strength of this study is that it was prospective, and higher vitamin D doses were used than is commonly recommended. It is the first prospective study of the hypothesis of using vitamin D in an attempt to prevent the recurrence of autism in the high risk group of newborn siblings to our knowledge. The weaknesses are that it is a small number of subjects and it is uncontrolled. More research with larger numbers and a control group is necessary to show cause and effect. On the positive side, we found that the children in our study were all born within two weeks of the expected date of confinement and their weights were all within normal limits. There were no premature births, nor were there any small-for-dates children born in comparison with 11.4% and 3% respectively (46,47). Further, there were no pre-eclampsia cases in contrast to 2-8% worldwide incidence (48). One mother had gestational diabetes in her previous pregnancy and the gestational diabetes occurred again with this pregnancy. Studies report that pre-eclampsia may be a contributing factor to autism (37). Vitamin D has been reported to be effective in reducing the incidence of pre-eclampsia, small-for-dates children and gestational diabetes (36,49). No mother developed hypercalcemia, kidney stones, or reported any adverse effects.

Our preliminary findings showed a reduction in the recurrence rate of autism in newborn siblings compared to the reported recurrence rate in the literature of about 20% (50). If contributing factors to autism include neuroinflammation and oxidative stress, then, vitamin D may be able to reduce the incidence of autism through its ability to reduce inflammation and oxidative stress (16,17). In addition, vitamin D may also have a positive effect on serotonin and its effect on brain development (29,30,31). Further, if there is defective pruning of synapses contributing to autism, vitamin D may play a role in normal synaptic pruning, and thereby reduce the incidence of autism (25).

The two children in our study who developed autism as diagnosed by the PDDBI, both regressed following an infection. They both had passed the MCHAT as not having autism. Both of the two children who developed autism have improved with treatment. One has improved to the degree that the child no longer carries the diagnosis of autism, although the child currently is diagnosed with Attention Deficit Hyperactivity Disorder (ADHD). We wondered whether one factor contributing to regression may be infections because one child had Klebsiella cultured from his stool. Both children have had significant gastrointestinal difficulties. It does seem to be clear that there was a regression in both of these children after their illnesses.

Klebsiella pneumonia is one of the organisms that can contribute to otitis media in children (51). These pathological organisms may have central nervous system effects. This may include inflammation, including meningitis, in addition to its upper respiratory infection and its gastrointestinal effects (52). Klebsiella is one of the organisms that produce propionic acid, which has been reported to contribute to autism symptoms in rats when injected intracerebrally (53). Alfawaz et al. reports that Vitamin D was protective from autistic features induced in propionic acid-intoxicated rat pups (54). Klebsiella has been developing a resistance to antibiotics and has recently become increasingly prevalent worldwide (55).

Niehus and Lord discussed the issue of early medical history of children with autism spectrum disorders (56). They found that children with ASD were more likely to have significantly more ear infections. However, typically developing children had significantly more illness-related fevers. Finally, they stated there was a non-significant trend toward the ASD group having more chronic gastrointestinal problems. Thus, as suggested by Sandler et al, children with autism may regress following pathological infections of the gut (57).

Luswolda et al. studied five East African ethnic groups across the life cycle and found that 25(OH)D levels were 46 ng/ml (58). They chose this group to study because Homo sapiens were believed to originate from East Africa. They believe that this level of 46 ng/ml 25(OH)D represents what is our evolutionary established circulating 25(OH)D should be. The exposure of these groups was mostly due to the sun, but some was due to the fish they ingested. Thus, our "Western Society" may need a higher dose of vitamin D or an increased exposure to the sun to achieve the levels of vitamin D of our "ancestors".

Our preliminary impression is that the best outcome for the children of mothers who get pregnant is for the mothers to be vitamin D "replete" during the periconceptual period, that is, to have the optimal level of vitamin D at least two months before the mother gets pregnant. This approach is being followed by ForumP2i.com (59). We would also recommend that the infants and toddlers be supplemented with vitamin D.

CONCLUSIONS

This is a presentation of a hypothesis and a preliminary report with small numbers. However, to our knowledge, it is the first to report on a prospective study using vitamin D during pregnancy of mothers of children with autism and supplementation of their newborn infants and toddlers. The newborn siblings were at high risk of developing autism. Our preliminary findings support the hypothesis that vitamin D during pregnancy at adequate doses and supplementing their infants and toddlers might reduce the incidence of autism. We also found support for previous studies showing that vitamin D may reduce the incidence of preterm births, small-for-dates, and pre-eclampsia.

We recommend that a larger prospective study using amounts of vitamin D aimed to achieve a level of 25 hydroxy D3 in the range of 30-50 ng/ml with a control population be studied. However, there are ethical problems in identifying vitamin D deficiency in a control group and not treating it, so such a study may be difficult to conduct.

Abbreviations

AMPK/mTOR: Adenosine MonoPhosphate Kinase/mammalian Target of Rapamycin signaling pathway

ADHD: Attention Deficit Hyperactivity Disorder

ASD: Autism spectrum disorder

IRB: Institutional Review Board

LC-MS/MS: Liquid Chromatography Mass Spectrography Mass Spectrography

MCHAT: Modified Checklist for Autism in Toddlers

PAML: Pathology Associates Medical Laboratories

PDDBI: PDD = Pervasive Developmental Disorders. BI = Behavioral Inventory

ROS: Reactive Oxygen Species

TPH1: Tryptophan hydroxylase 1

TPH2: Tryptophan hydroxylase 2

VDRE: Vitamin D response element

Conflicts of Interest statement

The authors declare that they have no conflicts of interest.

Sponsor had no role in collection, analysis, and interpretation of data, in writing of the manuscript, and the decision to submit the manuscript.

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The parents provided written informed consent for publication. They were informed that neither they nor their child would be personally identified in any reports or publications that may result from this study. The test results on individuals are de-identified. No identifying details, images or videos from participants are used in this publication. The consent forms are held in the Evergreen Center, Inc. in the office of the principal investigator. Consent forms are available for review by the Editor-in-Chief.

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Table 1. Results of 25 OHD, MCHAT and PDDBI

Coded ID	25OHD#1	Calcium #1	25OHD #2	Calcium #2	MCHAT Pass/Fail	PDDBI Pass/Fail	Illness/GI Disposition	FINAL DX/*
	Reference range 30 - 150	Reference range 8.5 – 10.5	Reference range 30 - 150	Reference range 8.5 – 10.5				
1	26 ng/ml	9.2 mg/dL	41 ng/ml	8.6 mg/dL	Pass	Fail	Yes	Autism
2	22 ng/ml	9.6 mg/dL	61 ng/ml	8.7 mg/dL	Pass	Pass	No	No Autism
3	26 ng/ml	8.8 mg/dL	79 ng/ml	8.1 mg/dL	Pass	Pass	No	No Autism
4	27 ng/ml	9.3 mg/dL	44 ng/ml	8.9 mg/dL	Pass	Pass	No	No Autism
5	15 ng/ml	9 mg/dL	45 ng/ml	8.9 mg/dL	Pass	Pass	Yes	No Autism
6	19 ng/ml	9.1 mg/dL	31 ng/ml	8.6 mg/dL	Pass	Withdrew	Withdrew	Withdrew
7	59 ng/ml	7.9 mg/dL	77 ng/ml	9 mg/dL	Pass	Fail	Yes	ADHD
8	25 ng/ml	8.5 mg/dL	36 ng/ml	n/a **	Pass	Pass	No	No Autism
9	31 ng/ml	9.1 mg/dL	32 ng/ml	8.9 mg/dL	Pass	Pass	No	No Autism
10	32.1 ng/ml	9.0 mg/dL	n/a	n/a	Pass	Pass	No	No Autism
11	31 ng/ml	8.3 mg/dL	42 ng/ml	8.3 mg/dL	Pass	Pass	No	No Autism
12	8 ng/ml	8.6 mg/dL	28 ng/ml	8.7 mg/dL	Pass	Pass	No	No Autism
13	40 ng/ml	8.9 mg/dL	46 ng/ml	9.4 mg/dL	Pass	Pass	No	No Autism
14	47 ng/ml	9.3 mg/dl	n/a	n/a	Fail	Pass	No	No Autism
15	20 ng/ml	n/a	33 ng/ml	n/a	Pass	Pass	No	No Autism
16	74 ng/ml	8.5 mg/dL	39 ng/ml	9.4mg/dL	Pass	Pass	No	No Autism
17	26 ng/ml	9 mg/dL	55 ng/ml	9 mg/dL	Pass	Pass	No	No Autism
18	69 ng/ml	8.7 mg/dL	55 ng/ml	8.8 mg/dL	Pass	Pass	No	No Autism
19	34 ng/ml	9.4 mg/dL	28 ng/ml	8.8 mg/dl	Pass	Pass	No	No Autism
20	43 ng/ml	9 mg/dL	48.3 ng/ml	9.5 mg/dL	Fail	Pass	No	No Autism

*Final Dx- 18/19 passed/no autism = 95% or 5% recurrence rate compared to the 20% recurrence rate reported in the literature

** n/a indicates not available