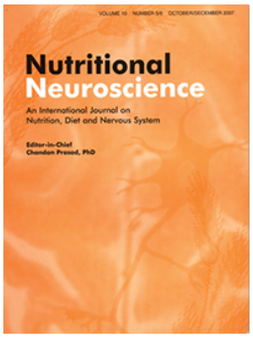

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# Fluctuations in clinical symptoms with changes in serum 25(OH) vitamin D levels in autistic children: Three cases report

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Autism spectrum disorder (ASD) is a common neurodevelopmental disorder caused by complicated interactions between genetic and environmental factors. Clinical trials, including case reports, case–control studies, and a double-blinded randomized clinical study, have suggested that high-dose vitamin D3 regimens may ameliorate the core symptoms of ASD. Vitamin D3 supplementation was effective in about three-quarters of children with ASD. To further investigate the relationship between vitamin D and ASD symptoms in vitamin D-responsive autistic children, changes in symptoms were assessed in three children with ASD who were given vitamin D3 supplementation followed by a long interruption. The core symptoms of ASD were remarkably improved during the vitamin D3 supplementation period when serum 25-hydroxyvitamin D [25(OH)]D levels reached over 40.0 ng/mL. However, symptoms reappeared after the supplementation was stopped, when serum 25(OH)D levels fell below 30.0 ng/mL but were again improved with re-administration of vitamin D3 after the interruption, when serum 25(OH)D levels exceeded 40.0 ng/mL. Overall, these results showed that the core symptoms of ASD fluctuated in severity with changes in serum 25(OH)D levels in children, indicating that maintaining a responsive 25(OH)D level is important for treating ASD. Maintaining a serum 25(OH)D level between 40.0 and 100.0 ng/ml may be optimal for producing therapeutic effects in vitamin D-responsive individuals with ASD.

**Keywords:** Autism, Vitamin D, Clinical trial, Children

## Introduction

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder caused by complicated interactions between genetic and environmental factors. The prevalence of ASD has steadily increased over the past three decades, from 1/2500 in prior reports to 1/68 in 2014.[1,2] Genetic factors, including gene mutations, copy number variations, and chromosomal abnormalities, and environmental factors, such as folic acid deficiency, antiepileptic drug use or food allergies, together contribute to the pathogenesis of ASD.[3–6] In addition, interactions between gene and environmental factors seem to play an important role in the etiology of ASD.[7,8] Recent studies have suggested that vitamin D3 supplementation could improve the core symptoms of ASD.[9–12] Vitamin D3 is relatively safe, readily available, and inexpensive, making it an attractive option for clinical application. The first case reported by our group suggested that vitamin D may directly ameliorate core autistic status; however, we emphasized that a single case was not representative of all ASD patients.[9] Later, two case-controlled, cross-sectional studies validated the efficacy of vitamin D supplementation in autistic children.[10,11] One of these two studies, undertaken by our group, indicated that vitamin D3 supplementation may significantly improve the core symptoms of ASD, especially in younger children.[10] A very recent double-blinded randomized clinical trial reported a positive core symptom response to a 16-week vitamin D3 supplementation period.[12] Vitamin D3 supplementation has been shown effective in approximately three-quarters of children with ASD.[13]

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However, no reports investigating the relationship between treatment efficacy and serum 25-hydroxyvitamin D [25(OH)]D levels have been published. Due to concerns over possible intoxication caused by high-dose vitamin D3 supplementation, parents or caregivers of children may discontinue vitamin D3 administration despite its effectiveness in ameliorating the core symptoms of ASD. In the present study, we investigated fluctuations in autistic symptoms in three children with ASD who were responsive to vitamin D3 supplementation. The supplementation was followed by a long interruption. The possible relationship between treatment efficacy and serum 25(OH)D level was also discussed.

## Patients and methods

This study was approved by the First Hospital of Jilin University, Changchun, China. The approved number of ethical committee was 2013-192, on 10 October 2013. The protocol was conducted in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans in 2000. Participants' caregivers were informed of the intervention composition, the safety profile, and the study requirements. All caregivers of all patients have given their informed written consent in accordance with the First Hospital of Jilin University. The 25OHD was measured by Guangzhou KingMed Center Clinical Laboratory, which is involved in the Vitamin D Standardization Certification Program (VDSCP) initiated by Center for Disease Control and Prevention (CDC), using the method of liquid chromatography and tandem mass spectrometry (LC-MS/MS) with the standard reference material 2972.

## Patient presentation

Three children with ASD were evaluated after referral to the First Hospital of Jilin University, and all three were diagnosed according to the DSM-V criteria of the American Psychiatric Association. The Autism Behavior Checklist (ABC, score for normal children <53) and the Childhood Autism Rating Scale (CARS, score for normal children < 30) were used to assess the severity of autism symptoms.[14,15] The CARS assessment was performed by an experienced neurodevelopmental pediatrician through observations of the children's behavior during the psychiatric examination, and the ABC evaluation was performed through parent interviews. The same neurodevelopmental pediatrician conducted both assessments.

None of the three patients had received any calcium and/or vitamin D therapy in the 6 months prior to joining the study. In addition, none of the children had a concomitant infection, showed photosensitivity or used photoprotection (such as broad-spectrum sunscreens) or other treatments known to affect serum 25(OH)D levels (such as antiepileptic medication, corticosteroids, or other immunosuppressive drugs). The family history of psychiatric illness was negative for all patients. Brain computed tomography scanning was normal, and karyotype analysis revealed no abnormalities (46XY). Metabolism screening in serum and urine was also normal. Serum 25(OH)D levels were tested, and the ABC and CARS assessments performed monthly.

The first patient was a 38-month-old boy. His serum 25(OH)D levels and assessment scores for autism symptoms are listed in Table 1. At baseline, the boy had an ABC score of 56 and a CARS score of 32. His serum 25(OH)D level was 11.9 ng/ml, as assessed by high-performance liquid chromatography. His basal serum calcium level was 2.35 mmol/L. Vitamin D3 was intramuscularly administered at a dosage of 150 000 IU in the hospital every month. At the same time, vitamin D was orally administered at a dosage of 800 IU/day under parental supervision. A follow-up evaluation was performed after one month. His parents reported a significant improvement in his behavioral problems. Laboratory tests showed that the child's serum 25(OH)D level increased to 46.7 ng/ml and serum calcium level was 2.30 mmol/L. ABC and CARS reassessments showed respective scores of 41 and 22. When he stopped taking vitamin D3 after 2 months due to concerns over possible vitamin D intoxication, his behavioral problems worsened. After the boy's 25(OH)D level dropped from 46.7 to 29.1 ng/ml, his total ABC and CARS scores increased to 59 and 32, respectively. Following this, vitamin D supplementation was resumed according to the schedule mentioned above. After one month of treatment, the serum calcium level was 2.38 mmol/L and his total ABC score (59 before and 35 after) and total CARS score (32 before and 24 after) were significantly reduced.

The same pattern was observed for the other two ASD patients, a 19-month-old boy and a 48-month-old boy. Their core symptoms, including symptoms related to social skills and language, body and object use, and self-help, were improved in accordance with the increased serum 25(OH)D level after vitamin D supplementation. Similarly, their symptoms became worse following the decrease in serum 25(OH)D after ceasing vitamin D supplementation (see Tables 1 and 2). The serum calcium levels remained in the normal range values during vitamin D treatment.

## Discussion

The three cases in this study suggested that the core symptoms of ASD fluctuate with changes in serum

**Table 1 The patients' serum 25(OH)D levels and autism symptom scores**

| Patients | 1 | | | | 2 | | | | 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | Male | | | | Male | | | | Male | | | |
| Age (months) | 38 | | | | 19 | | | | 48 | | | |
| Detection month | Jan 2014 | Feb 2014 | May 2014 | June 2014 | June 2014 | July 2014 | Sep 2014 | Oct 2014 | June 2014 | July 2014 | Oct 2014 | Nov 2014 |
| 25(OH)D level (ng/ml) | 11.9 | 46.7 | 29.1 | 43.7 | 9.6 | 38.4 | 27.2 | 49.4 | 22.0 | 55.0 | 28.0 | 42.4 |
| Total ABC score | 56 | 41 | 59 | 35 | 71 | 35 | 56 | 27 | 62 | 27 | 36 | 30 |
| Total CARS score | 32 | 22 | 32 | 24 | 38 | 29 | 36 | 27 | 28 | 24 | 27 | 25 |

25(OH)D levels. Our results demonstrate that the core ASD symptoms in children are improved after vitamin D supplementation. Therefore, vitamin D3 may play a vital role in the pathophysiology of ASD, and clinical assessment of vitamin D3 deficiency and subsequent supplementation, if needed, are critical.

To the best of our knowledge, this is the first report demonstrating that the core symptoms of ASD fluctuate in relation to serum levels of vitamin D based on direct measurements of serum 25(OH)D. In 2008, Cannell first hypothesized that vitamin D has a possible role in ASD.[16] An increasing number of studies have demonstrated that vitamin D deficiency is more prevalent in children with autism than in children showing typical development.[17,18] Wang *et al*. performed a meta-analysis of 11 studies evaluating a total of 870 patients and 782 healthy controls. The results showed that individuals with ASD had significantly lower levels of vitamin D than control subjects.[19] In addition, environmental data showed that reduced exposure to solar ultraviolet B increased ASD prevalence.[20–22]

Accumulating studies demonstrated that vitamin D was involved in numerous brain bioprocesses including neurotrophism, neuroimmunodulation, and neurotransmission and played a crucial role in brain functions.[23,24] Therefore, vitamin D could ameliorate the core clinical symptoms of ASD through several mechanisms such as antioxidant, brain protection, immune modulation, modifying the genetic mutation, and elevating brain serotonin content.[25]

25(OH)D is an index for assessing the nutritional status of vitamin D due to its biological stability and long half-life (2–3 weeks). The present criteria for vitamin D nutrition status were mainly formulated on the basis of bone health, with a particular focus on preventing rickets in children. However, vitamin D is also intimately related to the functioning of the central nervous system. Indeed, children with rickets often develop neuropsychiatric symptoms, such as night terrors, crying, and emotional problems. Thus, the brain may need relatively high serum vitamin D levels to maintain its proper biological function. None of the three children reported in this paper displayed any significant signs of rickets. Importantly, the children's ASD symptoms improved when their 25(OH)D levels reached 46.7, 38.4, and 55.0 ng/ml, and there were no adverse symptoms associated with the vitamin D supplementation. Unfortunately, their behavioral problems worsened following the discontinuation of vitamin D, as their 25(OH)D levels dropped to 29.1, 27.2, and 28.0 ng/ml. This result demonstrated that a 25(OH)D level below 30.0 ng/ml could not maintain the efficacy of the vitamin D response in children with ASD and that maintaining a 25(OH)D level above 40.0 ng/ml is essential for maintaining a therapeutic effect. According to the present criteria for vitamin D nutritional status, which suggest that a range of 20.0–100.0 ng/ml is adequate,[26,27] maintaining a serum 25(OH)D level between 40.0 and 100.0 ng/ml may be optimal for ensuring a therapeutic effect in vitamin D-responsive

**Table 2 Changes in the scores of the five subscales of the ABC**

| Patients | 1 | | | | 2 | | | | 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Detection month | Jan 2014 | Feb 2014 | May 2014 | June 2014 | June 2014 | July 2014 | Sep 2014 | Oct 2014 | June 2014 | July 2014 | Oct 2014 | Nov 2014 |
| Sensory | 6 | 3 | 6 | 3 | 15 | 8 | 12 | 6 | 9 | 6 | 6 | 6 |
| Social skills | 10 | 10 | 10 | 6 | 16 | 10 | 13 | 6 | 13 | 6 | 9 | 7 |
| Body and object use | 7 | 7 | 9 | 4 | 13 | 5 | 11 | 5 | 14 | 4 | 6 | 4 |
| Language | 15 | 11 | 17 | 10 | 9 | 5 | 6 | 2 | 16 | 9 | 13 | 11 |
| Social or self-help | 18 | 10 | 17 | 12 | 18 | 7 | 14 | 8 | 10 | 2 | 2 | 2 |

individuals with ASD. Because ASD symptoms can reappear when serum 25(OH)D levels drop, we recommend long-term continuation of vitamin D therapy for at least 6 months to a year, as long as a positive response is observed. Further clinical studies pinpointing the optimal therapy duration are warranted.

In conclusion, administration of high-dose vitamin D is effective in ameliorating the core symptoms of ASD, especially in younger children. These results were demonstrated in both the current paper and our previous studies. Maintaining a serum 25(OH)D level between 40.0 and 100.0 ng/ml may be optimal for achieving a therapeutic effect in vitamin D-responsive children with ASD

**Clinical Trial Registration**: Registry name: The associate of polymorphisms of vitamin D metabolism-related genes with autism, and the treatment of autism with vitamin D. Registration number: ChiCTR-CCC-13004498 http://www.chictr.org.cn/showprojen.aspx?proj=5074.

## Disclaimer statements

**Contributors:** LS, BW, HL, JF and ZX followed the patients and analyzed the data. K.S. and F.J. designed the study and drafted the manuscript. All authors were involved in the critical analysis of the final version of the manuscript. All authors approved the manuscript as submitted and agree to be accountable for all aspects of the work.

**Conflict of interest**: None.

**Ethics approval:** None.

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