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Selected vitamin D metabolic gene variants and risk for autism spectrum disorder in the CHARGE Study☆

Rebecca J. Schmidt [a,b,*], Robin L. Hansen [b,c], Jaana Hartiala [d], Hooman Allayee [d], Jaime L. Sconberg [e], Linda C. Schmidt [f], Heather E. Volk [g], Flora Tassone [b,f]

[a] Department of Public Health Sciences, University of California Davis School of Medicine, Davis, CA, USA
[b] Medical Investigation of Neurodevelopmental Disorders (M.I.N.D.) Institute, University of California Davis, Sacramento, CA, USA
[c] Department of Pediatrics, University of California Davis School of Medicine, Davis, CA, USA
[d] Department of Preventive Medicine, Institute for Genetic Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA, USA
[e] Graduate Group in Epidemiology, University of California Davis, Davis, CA, USA
[f] Department of Biochemistry and Molecular Medicine, University of California Davis School of Medicine, Davis, CA, USA
[g] Departments of Preventive Medicine and Pediatrics, Zilkha Neurogenetic Institute, Keck School of Medicine, Children's Hospital Los Angeles, University of Southern California, Los Angeles, CA, USA

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ABSTRACT

Background: Vitamin D is essential for proper neurodevelopment and cognitive and behavioral function. We examined associations between autism spectrum disorder (ASD) and common, functional polymorphisms in vitamin D pathways.
Methods: Children aged 24–60 months enrolled from 2003 to 2009 in the population-based CHARGE case–control study were evaluated clinically and confirmed to have ASD (n = 474) or typical development (TD, n = 281). Maternal, paternal, and child DNA samples for 384 (81%) families of children with ASD and 234 (83%) families of TD children were genotyped for: *TaqI*, *BsmI*, *FokI*, and *Cdx2* in the vitamin D receptor (*VDR*) gene, and *CYP27B1* rs4646536, *GC* rs4588, and *CYP2R1* rs10741657. Case–control logistic regression, family-based log-linear, and hybrid log-linear analyses were conducted to produce risk estimates and 95% confidence intervals (CI) for each allelic variant.
Results: Paternal *VDR TaqI* homozygous variant genotype was significantly associated with ASD in case–control analysis (odds ratio [OR] [CI]: 6.3 [1.9–20.7]) and there was a trend towards increased risk associated with *VDR BsmI* (OR [CI]: 4.7 [1.6–13.4]). Log-linear triad analyses detected parental imprinting, with greater effects of paternally-derived *VDR* alleles. Child *GC* AA-genotype/A-allele was associated with ASD in log-linear and ETDT analyses. A significant association between decreased ASD risk and child *CYP2R1* AA-genotype was found in hybrid log-linear analysis. There were limitations of low statistical power for less common alleles due to missing paternal genotypes.
Conclusions: This study provides preliminary evidence that paternal and child vitamin D metabolism could play a role in the etiology of ASD; further research in larger study populations is warranted.

1. Introduction

Autism spectrum disorder (ASD) consists of a range of neurodevelopmental disorders characterized by the presence of social deficits, language impairments, and stereotyped or repetitive behaviors and interests. The etiology of ASD in most cases remains unclear, though combinations of multiple genetic and environmental factors are likely to play a role. Vitamin D deficiency was hypothesized to contribute to the increase in the incidence of ASD based on studies showing increased rates of autism among dark-skinned immigrants displaced into northern latitudes, and differences in autism prevalence across season and latitude [1], potentially reflecting changes in sunlight exposure and absorbed vitamin D. These findings have been attributed to a potential effect of *maternal* vitamin D status on the child's risk for ASD. The biologic plausibility for a link between vitamin D and autism is ample, as previously reviewed [1,2]. Animal studies show long-lasting neurodevelopmental effects of transient vitamin D deficiency during gestation leading to autism-relevant structural and functional changes

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* Corresponding author at: 123 Medical Sciences, 1C One Shields Ave., Davis, CA 95616-8638, USA. Tel.: +1 530 752 3226; fax: +1 530 752 3239.
E-mail addresses: rjschmidt@ucdavis.edu (R.J. Schmidt), rlhansen@ucdavis.edu (R.L. Hansen), hartiala@usc.edu (J. Hartiala), hallayee@usc.edu (H. Allayee), jlsconberg@ucdavis.edu (J.L. Sconberg), SchmidtLC@aol.com (L.C. Schmidt), hvolk@usc.edu (H.E. Volk), ftassone@ucdavis.edu (F. Tassone).

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in the brain and behaviors of the offspring [3–5]. Gene variants within the vitamin D pathway can determine uptake and utilization of vitamin D. Genetic susceptibility to inefficient vitamin D uptake and metabolism has yet to be explored in relation to autism. Thus, this study examined common, functional vitamin D-relevant gene variants in maternal, paternal and child samples in relation to risk for ASD in the child.

2. Methods

2.1. Participants, eligibility and diagnostic criteria

Individuals included in this study were participants in the CHARGE (*CH*ildhood *A*utism *R*isks from *G*enetics and the *E*nvironment) case–control study [6]. Eligibility criteria for children were: 1) age of 24 to 60 months at time of enrollment, 2) birth in California, 3) residence with at least one biologic parent who speaks English or Spanish, and 4) residence in the catchment areas of a specified list of California Regional Centers that coordinate services for persons with developmental disabilities. Children with autism, intellectual disability, or developmental delay were identified through the California Regional Centers as having received services for one or more of these conditions. Autism cases were also referred from the MIND Institute and other health or service providers, or self-referred from the CHARGE Study website. General population controls identified from state birth files were frequency matched to the age and catchment area distribution of the autism cases, and a 4:1 male-to-female ratio reflective of that seen for ASD.

All children were assessed for cognitive function using the Mullen Scales of Early Learning (MSEL) [7] and for adaptive function using the Vineland Adaptive Behavior Scales (VABS) [8]. For children with autism, the primary caregiver completed the Autism Diagnostic Interview — Revised (ADI-R) [9], and children were assessed using the Autism Diagnostic Observation Schedule — Generic (ADOS) [10] to confirm the child's diagnosis. The children of families recruited from the general population or with developmental delay/intellectual disability were screened for evidence of ASD using the Social Communication Questionnaire (SCQ) and if they scored above 15, were evaluated for autism. Final ASD case status was defined as 1) scoring at least 7 on ADOS Module 1 or at least 8 on ADOS Module 2; 2) meeting the cutoff value for section A or B and scoring above or within 2 points of the cutoff value on A or B (whichever did not meet cutoff value) in ADI-R; and 3) meeting the cutoff value on section D in ADI-R. Typical development (TD) required being recruited from the general population, screening negative for evidence of ASD on the SCQ, and scoring 70 or above on both the MSEL and VABS. These analyses included only the first child per family recruited into the study. The University of California—Davis Institutional Review Board and the State of California Committee for the Protection of Human Subjects approved this study and the CHARGE Study protocols. Neither data nor specimens were collected until written informed consent was obtained from the parents.

2.2. Genotyping methods

Genomic DNA was isolated using standard procedures (Puregene kit; Gentra Inc.) from peripheral blood plasma leukocytes collected as part of CHARGE protocol. Genotyping was conducted blinded to case status using TaqMan Single Nucleotide Polymorphism (SNP) Genotyping Assays (Applied Biosystems) for the following variants: *VDR TaqI* (rs731236), *BsmI* (rs1544410), *Cdx2* (rs11568820), and *FokI* (rs10735810), *CYP27B1* T2838C (rs464536), *GC* (*VBP*, *DBP*) G716A (rs4588), and *CYP2R1* rs10741657. Variants were chosen from key regulatory genes for the pathway of interest with priority given to common variants that altered gene function and/or were associated with altered vitamin D status.

Ancestry Informative Markers (AIMs) were also genotyped for a subset of participants, including 281 (73%) families of children with ASD, and 161 (69%) families of children with TD. We identified 100 SNPs based on inherited allele frequencies determined from four parental populations (African, European, American Indian, and East Asian) to empirically estimate the proportion of ancestry attributable to a particular founding population for each individual using the program Structure. In our analyses, the proportion of variance from the European group was used as a reference with the additional three variables reflecting ancestral heritage included as covariates.

2.3. Statistical analysis

2.3.1. Case–control logistic regression models

Odds ratios (OR) and 95% confidence intervals (CI) were estimated for associations between the gene variants and ASD, adjusted for confounders, using logistic regression analysis applied to a case–control design using SAS 9.4. Potential confounders included: maternal, paternal, and child race and ethnicity (self-reported by parents, derived for child from parental information), private insurance vs. public payment for delivery, maternal and paternal age, maternal birthplace (US, Mexico, other), education, pre-pregnancy body mass index, and child sex and birth year. Ancestral heritage derived from the AIMs was also examined as potential confounders on the subset of participants with this data available (earlier participants). We began by fitting a full model containing potential confounders identified in the bivariate analyses as being broadly associated ($P < 0.2$) with both ASD and each genetic variant. Variables were then excluded using backward selection, retaining in the model variables that caused ≥ 10% change in the parameter estimates for the gene variants of interest. Because biologic samples were not available for some participants and many fathers, sensitivity analyses assessed the impact of missing data, using multiple imputation via the Markov Chain Monte Carlo algorithm [11]. To account for the multiplicity of hypotheses being assessed, we controlled the false discovery rate (FDR) at 5% [12].

Interaction effects were examined between gene variants and race and ethnicity, parental age, maternal birthplace, pre-pregnancy body mass index, and child sex. In addition, because nutrient data from vitamins, supplements and cereals for the three months before and during pregnancy was available for these study participants, we conducted exploratory analyses examining multiplicative interactions between maternal supplemental vitamin D intake above and below the mean/median for the TD group (400 IU) and child gene variants that were associated with increased risk for ASD in the case–control or hybrid log-linear analyses, and that were not vulnerable to population structure bias.

2.3.2. Linear regression models

Linear regression models were fit for associations between genotypes and continuous assessment scores in secondary (post-hoc) analyses. The multiple linear regression coefficients represent adjusted mean differences in continuous scores across genotype. The continuous assessment scores included: age-standardized MSEL composite score, VABS composite score, SCQ total score, and the ADOS-2 comparison (severity) score [13]. T-tests were also preformed to compare mean assessment scores by genotype.

2.3.3. Log-linear models

Log-linear analysis was applied to both a case–parent triad design [14] and, when population structures allowed, a hybrid design that combined the case–parent triad and case–control designs [15].

2.3.3.1. Case–parent triad log-linear. The case–parent log-linear approach was used to assess the association between ASD and both maternal and infant genotypes with the case–parent triad as the unit of analysis [14]. This approach provided likelihood-ratio tests (LRTs) and maximum-likelihood estimators of the genetic effects, allowing for different relative risks (RRs) corresponding to carrying one and carrying two copies of a susceptibility-related allele, relative to no copies [14]. The