

DISEASES AND DISORDERS

Dynamical features in fetal and postnatal zinc-copper metabolic cycles predict the emergence of autism spectrum disorder

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Metals are critical to neurodevelopment, and dysregulation in early life has been documented in autism spectrum disorder (ASD). However, underlying mechanisms and biochemical assays to distinguish ASD cases from controls remain elusive. In a nationwide study of twins in Sweden, we tested whether zinc-copper cycles, which regulate metal metabolism, are disrupted in ASD. Using novel tooth-matrix biomarkers that provide direct measures of fetal elemental uptake, we developed a predictive model to distinguish participants who would be diagnosed with ASD in childhood from those who did not develop the disorder. We replicated our findings in three independent studies in the United States and the UK. We show that three quantifiable characteristics of fetal and postnatal zinc-copper rhythmicity are altered in ASD: the average duration of zinc-copper cycles, regularity with which the cycles recur, and the number of complex features within a cycle. In all independent study sets and in the pooled analysis, zinc-copper rhythmicity was disrupted in ASD cases. In contrast to controls, in ASD cases, the cycle duration was shorter ($F = 52.25$, $P < 0.001$), regularity was reduced ($F = 47.99$, $P < 0.001$), and complexity diminished ($F = 57.30$, $P < 0.001$). With two distinct classification models that used metal rhythmicity data, we achieved 90% accuracy in classifying cases and controls, with sensitivity to ASD diagnosis ranging from 85 to 100% and specificity ranging from 90 to 100%. These findings suggest that altered zinc-copper rhythmicity precedes the emergence of ASD, and quantitative biochemical measures of metal rhythmicity distinguish ASD cases from controls.

INTRODUCTION

Autism spectrum disorder (ASD) affects 1 to 2% of the population in developed countries. Reports have shown that multiple nutrient elements and toxic metals are differentially absorbed and metabolized in children with ASD (1–3). However, the mechanisms underlying elemental dysregulation and ASD risk are not well understood, and it is not known whether this elemental dysregulation is present in fetal and early postnatal life before first clinical symptoms manifest.

Here, we tested whether zinc-copper pathways were disrupted in ASD and whether quantitative measures of zinc-copper dynamics could provide a biochemical basis to distinguish ASD cases from controls. We selected zinc-copper cycles as our primary target for testing the dysregulation hypothesis because these are highly conserved in evolution, are essential for maintenance of health, and also exert homeostatic control over known neurotoxins (4). Notable examples of disorders arising from perturbations of the metabolism of these metals include Wilson's disease, a key component of which is copper dysregulation that leads to progressive neurological and cognitive dysfunction and psychosis-like symptoms also documented in adults with ASD (5). In Wilson's disease, realigning the zinc-copper interaction via zinc supplementation increases the expression of the metal-binding protein metallothionein, which helps regulate copper levels (6). Recently, zinc-associated pathways have been implicated in ASD (4, 7, 8). For example, mutations in the gene coding for *SHANK3*, which is part of a zinc-mediated signaling system, are linked to increased ASD risk (9).

Many physiologic processes follow cyclic patterns or rhythms that operate at a wide range of time intervals, from milliseconds (for example, neuron firing) to hours (for example, body temperature), days or circadian (for example, sleep cycles), weeks, or longer (for example, menstrual cycles) (10, 11). For any system in the body, multiple cycles operating at different timescales may interact, and the study of these processes requires methods that focus not solely on concentration but rather on the dynamic changes over time (12, 13). Zinc and copper levels are not stationary in the human body, exhibiting a cyclic pattern (14–16). We therefore focused on their cyclic properties rather than single-point measures of serum concentrations. We use the term cycles to indicate time-dependent rhythmic variation in the concentration of

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