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Full-length Article

General intelligence is associated with subclinical inflammation in Nepalese children: A population-based plasma proteomics study

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ABSTRACT

Improving child cognition in impoverished countries is a public health priority. Yet, biological pathways and associated biomarkers of impaired cognition remain poorly understood and largely unknown, respectively. This study aimed to explore and quantify associations between functional plasma protein biomarkers and childhood intellectual test performance. We applied proteomics to quantify proteins in plasma samples of 249 rural Nepalese children, 6–8 years of age who, 1 year later at 7–9 years of age, were administered the Universal Nonverbal Intelligence Test (UNIT). Among 751 plasma proteins quantified, 22 were associated with UNIT scores, passing a false discovery rate threshold of 5.0% ($q < 0.05$). UNIT scores were higher by 2.3–9.2 points for every 50% increase in relative abundance of two insulin-like growth factor binding proteins (IGFBPs), six subclasses of apolipoprotein (Apo) and transthyretin, and lower by 4.0–15.3 points for each 50% increase in relative abundance of 13 proteins predominantly involved in inflammation. Among them, IGFBP-acid labile subunit, orosomucoid 1 (ORM1), Apo C-I, and pyruvate kinase isoenzymes M1/M2 jointly explained 37% of the variance in UNIT scores. After additional adjustment for height-for-age Z-score and household socio-economic status as indicators of long-term nutritional and social stress, associations with 6 proteins involved in inflammation, including ORM1, α-1-antichymotrypsin, reticulocalbin 1, and 3 components of the complement cascade, remained significant ($q < 0.05$). Using untargeted proteomics, stable, constitutive facets of subclinical inflammation were associated with lower developmental test performance in this rural South Asian child population. Plasma proteomics may offer opportunities to identify functional, antecedent biomarkers of child cognitive development.

1. Introduction

As child survival improves (The United Nations, 2015), attention is turning to assessing and alleviating coexisting burdens of stunted growth and suboptimal cognition among children in low and middle income countries (Black et al., 2013; Grantham-McGregor et al., 2007). Both conditions can be a consequence of undernutrition (Black et al., 2013; Walker et al., 2011) and share complexity and incomplete knowledge of their etiologies, challenging effective prevention (Gale, 2005). Yet, while the Sustainable Development Goals agenda for the year 2030 remains focused on reducing childhood growth faltering (The United Nations, 2014), similar multi-sectoral efforts to quantify the burden and develop interventions to prevent impaired cognition are lagging.

Difficulty and costs in deploying standardized tests across cultures represent clear obstacles to advancing the child development agenda (Prado et al., 2010). In addition, however, there remains population data on the complex biosocial interactions and risk factors of impaired cognition that could assist in identifying groups at risk (Turkheimer et al., 2003; Walker et al., 2007). Chronic exposure to protein-energy and micronutrient deficiencies (i.e., iron and iodine), infectious agents, environmental hazards, social stresses, and lack of stimulation and learning opportunities, all deeply rooted in poverty, have been identified as risk factors (Walker et al., 2011), but leaving the question how these insults disturb

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long-term cumulative biological processes of brain development unanswered.

During the past decade, emerging "-omics" studies have contributed to exploring biological pathways and discovering molecular footprints associated with neurodevelopmental disorders (Antonarakis et al., 2004; Bahado-Singh et al., 2013; Corbett et al., 2007; Woods et al., 2015). The high-throughput technologies are powerful in that they do not require prior knowledge, thereby enabling unprecedented discovery and allow analysis of hundreds of biological molecules with a single assay, providing comprehensive insights into the multifactorial nature of cognitive deficits. These advances, however, have yet to be applied to improve our understanding of common biological pathways that underlie suboptimal development of cognitive function of children in the developing world, where the most serious loss of developmental potential occurs (Grantham-McGregor et al., 2007).

The plasma proteome which is currently being explored for its potential to advance assessment of nutritional and health status of children (Cole et al., 2013; Lee et al., 2015; West et al., 2015) may provide a novel opportunity to identify biomarkers of molecular networks of homeostatic systems or other environmental exposures that affect cognitive function of children. The plasma protein milieu exhibits relative constancy within individuals over time and reflects a systemic "sum" of tissue status in the body (Anderson and Anderson, 2002). Neurotrophic and neuroendocrine factors that play key roles in neuronal cell signaling, synaptic plasticity and neurogenesis are present in plasma (Iughetti et al., 2011; O'Dorisio et al., 2002). In addition, the plasma proteome contains proteins whose expression are influenced by genetics, age, gender and nutritional and health status that are known to be underlying contributing factors of brain function (Dauncey, 2009). Plasma proteins influenced by some of these risk factors may not directly affect brain function, but can still indicate cognitive function as a systemic response to conditions that affect both protein abundance in plasma and developmental processes in the central nervous system (CNS).

In this study, we hypothesized that proteins that directly mediate or indirectly reflect cognitive function can be detected and quantified in plasma, and thus provide a repertoire of biomarkers that could provide insight into risk factors of poor cognition within or between populations. In southern Nepal, as part of a follow-up study in the offspring of women who participated in a prenatal micronutrient supplementation trial, we collected plasma samples at 6–8 years of age (Schulze et al., 2014; Stewart et al., 2009b). A year later, intellectual and motor functioning were assessed by standardized tests in a subgroup of the children at 7–9 years of age (Christian et al., 2010) at the time when the brain for higher-cognitive functions continues to mature (Grantham-McGregor et al., 2007). Using existing plasma samples and applying quantitative proteomics, we sought to identify plasma proteins that co-varied with cognition a year later, reflected by child performance on a standardized test of general intelligence.

2. Methods

2.1. Study design, population, and subjects

Children assessed in this study were a subset of a cohort of 4130 children born to 4926 mothers in the southern plains district of Sarlahi, Nepal who participated in a randomized controlled trial of antenatal micronutrient supplementation from 1999 to 2001 (ClinicalTrials.gov:NCT0011527) (Christian et al., 2003a,b). Of these, 3673 children subsequently (2001–2004) participated in a second trial that evaluated growth effects of daily iron and/or zinc supplementation from 6 to 36 months of age, (Tielsch et al., 2006) forming part of their history of nutritional exposure. In 2006–2008, 3524 children in this cohort were re-assessed at 6–8 years of age for nutritional and health status, that included blood sample collection (Stewart et al., 2009a,b). Children with available plasma samples meeting inclusion criteria (n = 2130), were stratified by the five antenatal supplementation groups, each from which 200 children randomly selected for in-depth biochemical biomarker assessment (total n = 1000) (Schulze et al., 2014). From these, plasma samples of 100 (50%) children from each of 5 maternal micronutrient supplementation groups (total n = 500) were randomly selected for mass spectrometric proteomics analysis (Cole et al., 2013).

One year after the 2006-8 assessment, ~1900 of the 3524 children were followed for neurocognitive and motor function testing, at 7–9 years of age (Christian et al., 2010). Thus, the present analysis was carried out on a subset of 251 children from the original trial whose records have both plasma proteomics data at 6–8 years of age and intelligence test data at 7–9 years (Fig. 1). Two children whose plasma samples appeared to be as a single sample within a mass spectrometry experiment were further excluded, yielding 249 children for analysis. Children in the present study (n = 249) were comparable based on numerous characteristics to other children in the proteomics study who were not assessed for cognitive function (also n = 249), except for a 0.6 kg heavier weight ($P = 0.022$) (data not shown). Participation in all studies required parental informed consent and all protocols were reviewed and approved by institutional review boards in Kathmandu, Nepal and Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

2.2. Assessment of general intelligence

Children's intellectual functioning was assessed using the Universal Nonverbal Intelligence Test (UNIT). The UNIT was designed to provide a measure of general intelligence in a completely nonverbal format for children who would be unfairly assessed with a language-loaded ability test (Bracken et al., 1998). It measures mainly memory and reasoning abilities consisting of six subtests: symbolic, spatial, and object memory, analogic reasoning, cube design, and mazes. Details about the administration of the UNIT and the standardization of raw scores have been reported elsewhere (Christian et al., 2010). The analogic reasoning subtest was removed because it was not culturally appropriate to Nepalese children. Because this test has not been standardized in Nepal, factor structure was assessed using exploratory and confirmatory factor analyses. Total scores of the raw scores of five subtests were generated and were converted to *T*-scores (mean 50 and standard deviation 10) based on child's age.

2.3. Child nutritional and health assessments and household socio-economic interview

Nutritional, health, demographic and socio-economic status (SES) assessments during the follow-up surveys have been described elsewhere (Stewart et al., 2009a,b). Briefly, during home visits, trained staffs asked heads of household about children's years of schooling and household SES information (caste, religion, household asset ownership, parental education, and parental literacy). Anthropometrists measured children's height and weight by standard procedures and collected 7-day histories of morbidity symptoms and dietary intake (Stewart et al., 2009a). Plasma concentrations of retinol, 25(OH)D, cobalamin, folic acid, transferrin receptor, ferritin, and thyroglobulin were measured to assess child vitamin A, D, and B12, folate, iron, and iodine status. For iron status, transferrin receptor to ferritin ratio (TfR:ferr) was used (Malope et al., 2001). High sensitive C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP), two widely used inflammation makers for

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