Psychiatry Research 229 (2015) 1031–1037

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Increased homocysteine levels correlate with the communication deficit in children with autism spectrum disorder

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ARTICLE INFO

Article history:
Received 14 November 2014
Received in revised form
5 May 2015
Accepted 20 May 2015
Available online 29 May 2015

Keywords:
Autism spectrum disorder
Core symptoms
Metabolism
Homocysteine
Language development
Biomarker

ABSTRACT

The clinical significance of high levels of homocysteine in autism spectrum disorder (ASD) is unknown. An experimental study was conducted in order to evaluate the concentration of homocysteine in children with ASD and typically developing children and to analyse any relationships with the severity of core symptoms of ASD and other clinical features (drugs, co-morbidities, gender, age, diet). Core symptoms of autism were evaluated by DSM-IV criteria. Homocysteine, glutathione, methionine, 3-nitrotyrosine were measured in urine. The increase in homocysteine concentration was significantly and directly correlated with the severity of the deficit in communication skills, but was unrelated to deficit in socialisation or repetitive/restricted behaviour. Urinary homocysteine concentration may be a possible biomarker for communication deficits in ASD and a potential diagnostic tool useful to evaluate new treatment options since no treatment for core symptoms of ASD are available.

1. Introduction

Autism spectrum disorder (ASD) encompasses a group of a neurobehavioral disorders characterised by abnormalities in three behavioural domains including social interaction, communication, and repetitive stereotypic behaviours. ASD affects approximately 1% of children and is on the rise (MMWR, 2010). There are significant genetic mechanisms underlying these disorders and research studies have uncovered several metabolic abnormalities associated with ASD (Guevara-Campos et al., 2013, 2015; Frye and Rossignol, 2014; West et al., 2014). Several clinical trials with different drugs aimed to reduce oxidative stress in ASD patients have been conducted (Frustaci et al., 2012) and the overall studies led to mixed results. The apparent discrepancies among studies in this research field may arise from many methodological shortcomings which needed to be clearly considered when analysing data such as the age, gender, autism symptoms severity, type of autism spectrum disorders, medical comorbidities, diet, concomitant medications, body mass index, and heterogeneity of analytical techniques (Ghanizadeh, 2013). A large clinical trial evaluated the effect of vitamin/minerals supplementation (20 vitamins plus 14 minerals) in patients with ASD (age 3–60 years old) and found that it improved autism symptoms measured by a revised form of the Parent Global Impressions in particular towards receptive language, hyperactivity, tantruming and overall/average effects (Adams et al., 2011). The treatment also reduced the concentration of oxidative markers i.e. nitrotyrosine and the ratio of oxidised glutathione to reduced glutathione in plasma (Adams et al., 2011). Other studies found alterations in some markers related to oxidative stress (i.e. reduced free glutathione and decrease in the ratio between reduced and oxidised glutathione) in blood of patients with ASD and no changes in other markers of oxidative stress such as 3-nitrotyrosine as reported here and in agreement with findings reported by another group (Frye et al., 2013a). A double-blind, placebo controlled study found a reduction of autism severity (measured by Ritvo-Freeman scale) after 30-week administration of high dose of vitamin C (110 mg/kg) (Dolske et al., 1993). Pilot study of the effect of methyl B12 treatment on behavioural and biomarker measures in children with ASD (age 3–8 years) showed no significant effect. However, administration of methyl B12 did improved symptoms in a subgroup of children with ASD (30%) (Bertoglio et al., 2010) suggesting

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http://dx.doi.org/10.1016/j.psychres.2015.05.021
0165-1781/

that within the diagnostic label of ASD, different patients may benefit from different therapeutic approaches. Open label trial showed that administration of methylcobalamin and folinic acid for 3 months improved autism scores measured by the Vineland Adaptive Behavior Scale (Frye et al., 2013b) as well as glutathione redox metabolites (Frye et al., 2013b; James et al., 2009). One of the metabolic alterations that have been often reported in ASD is an increase in the concentration of the sulphuric amino-acid, homocysteine. Homocysteine concentration is regulated by the so called "methionine cycle" which involves the regeneration of the aminoacid methionine from homocysteine via the B12-dependent transfer of a methyl group from 5-methyl-tetrahydrofolate via the methionine synthase reaction (Main et al., 2010 for review). Methionine is then activated to S-adenosylmethionine (SAM), the methyl donor for multiple cellular methyltransferase reactions. The transfer of the methyl group from SAM results in the demethylated product S-adenosylhomocysteine (SAH). The reversible hydrolysis of SAH to homocysteine completes the methionine cycle. Homocysteine can then be either remethylated to methionine or irreversibly removed from the methionine cycle by cystathionine beta synthase. This reaction is a one-way reaction that permanently clears homocysteine from the methionine cycle and initiates the trans-sulfuration pathway for the synthesis of cysteine and glutathione. The regulation of homocysteine concentration depends on several metabolic routes and enzymes and depends on availability of some form of folate and vitamin B12. A recent study performed in Omani children with ASD found a decreased vitamin B12 and folate concentration (Al-Farsi et al., 2013) however no significant alterations have been found in other studies (Adams et al., 2011; Lowe et al., 1981; Melnyk et al., 2012). Conversely, the expression of some of the vitamin B12- or folate-dependent enzymes involved in homocysteine metabolic routes has been shown to be altered in patients with ASD (Frustaci et al., 2012 for review). Interestingly, a recent meta-analyses demonstrated in patients with ASD, two functional polymorphisms in the gene of methylene tetrahydrofolate reductase, the enzyme that converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is needed for the remethylation of homocysteine to methionine (Frustaci et al., 2012). The C677T allele has been found with significantly higher frequencies in ASD patients in three studies, whereas the difference was not significant in the other three. After meta-analysis, the OR in homozygous mutant subjects (TT genotype), and in heterozygous mutant subjects (CT geno-type) compared with homozygous non mutant subjects (CC geno-type), was respectively 2.26 (95% CI 1.30–3.91) and 1.57 (95% CI 1.14–2.16) (Frustaci et al., 2012). Hyperhomocysteinemia causes brain dysfunction by two suggested mechanisms, namely, oxidative damage and abnormal DNA methylation (Ho et al., 2002; Perna et al., 2003). Several reports showed an increased homocysteine concentration in biological fluids in children/adolescents with ASD compared to age-matched TD controls (Paşca et al., 2006; Adams et al., 2011; Ali et al., 2011; Kałuzna-Czaplińska et al., 2011; Tu et al., 2012), however other failed to find such difference (James et al., 2004). Evaluation of the relationship between homocysteine and the clinical features and severity of core symptoms in patients with ASD may help to shed some light on the role of this unusual amino acid in the symptomatology of the disorder and ultimately to identify any possible links between specific neuropsychiatric alterations and the common pathophysiological changes present in this disease (Parellada et al., 2012; Gonzalez-Liencres et al., 2014). Finally, identifying differential biomarkers of a single core symptom of ASD could be very helpful in pinpointing specific mechanisms responsible for the phenotype and may help tailor new pharmacological for children with ASD with this biochemical alteration.

Therefore in this study we assessed three main objectives:

1) First, we aimed to replicate the findings reported in recent literature related to the homocysteine concentration increases in children with ASD. In addition we measured the concentrations of metabolites elated to homocysteine i.e. methionine, cysteine and glutathione.
2) Next, we evaluated the relationship between urine homocysteine levels and each of the three criteria of ASD according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV).
3) Finally we studied the influence of several confounding factors such as age, gender, past or current intestinal problems, food allergy or intolerance, sleep problems mainly difficulty in starting to sleep or early nocturnal awakening, vitamin intake (excluding vitamin B12), vitamin B12 supplementation, docosahexaenoic acid (DHA) supplementation, mineral intake, regression, prescription drug intake, antiepileptic drug intake, epilepsy, head circumference, body mass index, and being verbal or non-verbal.

2. Methods

2.1. Study design

An observational, cross-sectional study performed in Valencia (Spain) (between 2012 and 2013) in order to find suitable biomarkers of core symptoms of ASD. Clinical information was retrieved by reviewing the medical histories, by administering a questionnary/interview to parents and by evaluating of children behaviour by a Psychiatrist and a Psychologist.

2.2. Setting and participants

The study involved 69 children: 35 children with ASD (aged 4–13 years) and 34 neurologically healthy children (aged 4–12 years and sex-matched). Study participants were recruited from patients attending specialised and qualified centres for psychotherapeutic intervention in children with ASD. Neurologically healthy children (gender-matched) were recruited from public school. Data were collected between September 2012 and November 2013. All children had previously been diagnosed with ASD by a clinical psychologist/psychiatrist. ASD was confirmed at the time of the study by using the DSM-IV diagnostic criteria using a standard neurodevelopment examination and interview. In addition, all other relatively common genetic or neurological conditions responsible for syndromic ASD were ruled out. The entire study protocol was approved by the ethics committee at the University of Valencia (Spain) (protocol number H1397475950160). Written informed consent was obtained from all parents.

2.3. Evaluation of core symptoms of autism spectrum disorder (ASD)

All children were previously diagnosed with an ASD using the criteria of ICD-10 (tenth revision International Statistical Classification of Diseases and Related Health Problems) and the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, fourth edition.) Core symptoms of autism based on the DSM-IV were evaluated with the revised autism diagnostic interview™ (ADI-R). Cut off scores are 10 for social interaction, 8 for communication and language, if verbal, and 7 if non-verbal, and 3 for restricted and repetitive behaviours. This diagnostic tool has a high correlation level with the diagnostic criteria reported in the DSM-IV and has good psychometric properties (96% sensitivity and 92% specificity) (Lord et al., 1995).

2.4. Medical interview and evaluation

Extensive medical histories of the autistic and control children were taken. The parents were asked for: a detailed history of pregnancy and labour, birth morphometric measures, Apgar scores, vaccinations, mental and motor development, any illnesses or traumatic events, diet, body mass index, head circumference, vitamin or supplement intake, medication, epilepsy, gastrointestinal or sleep problems, regression, food allergies or intolerances, and laboratory results about syndromic causes of ASD (fragile X, chromosomopathy, genetic diseases, etc.).