

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Oral Ascorbic Acid and Alpha-Tocopherol to Reduce Behavioural Problems in Young Patients Affected of Fragile X Syndrome: A Randomized, Double-Blind, Placebo-Controlled Phase II Pilot Trial

Y. de Diego Otero¹ et al.,*

¹Research Laboratory, Fundacion IMABIS, Hospital Carlos Haya, Malaga,

²Unidad de Gestion Clinica de Pediatria, Hospital Carlos Haya, Malaga,

³INFOBIOTIC S.L., Malaga,

⁴IBGM, Universidad de Valladolid,

⁵Servicio de Pediatria, Hospital Quiron, Madrid,

⁶Unidad de Gestion Clinica de Salud Mental. Hospital Carlos Haya, Malaga, Spain

1. Introduction

Fragile X syndrome (FraX) was first described by Dr. Martin and Dr. Bell in 1943, in families with both males and females affected by sex-linked mental retardation (1) and was later identified as the most common cause of inherited mental retardation (2-4). The prevalence of the Fragile X syndrome has been estimated in 1 out of 2,500 males and 1 out of 4,000 females (5, 6).

In addition of moderate to severe mental retardation, FraX individuals exhibit macroorchidism, an elongated face (7), long ears, connective tissue dysplasia, hyperactivity, autistic-like and stereotypical behaviours, speech delay and increased sensory sensitivity (8,9). Typical neuropathological features of the FraX are long, thin, and tortuous appearance of cortical dendritic spines (10,11), increased intracranial volume (12), enlarged ventricles, increased volumes of selective subcortical gray matter regions, decreased size of the posterior cerebellar vermis (13), and an altered glucose metabolism (14).

The syndrome was named after identification of a fragile site that was located in the long arm of the X chromosome, detected by cytogenetic testing in a cell culture medium deprived of folic acid (15). The Fragile X mental retardation 1 (*FMR1*) gene was linked to that region (Xq27.3) and a dynamic CGG repeat expansion mutation was determined to be the cause of the syndrome (16). A full-mutation with more than 200 CGG repeats, causes methylation of the *FMR1* gene and consequently leads to a transcriptional silencing of the gene and the

* C. Quintero-Navarro¹, Rocio Calvo-Medina², R. Heredia-Farfan¹, L. Sanchez-Salido¹, E. Lima-Cabello¹, A. Higuero-Tapiador¹, I. del Arco-Herrera³, I. Fernandez-Carvajal⁴, T. Ferrando-Lucas⁵ and L. Perez-Costillas⁶

absence of the FMRP protein(17). It has been established as a normal range of CGG triplets between 6 and 55 repeats, and a CGG expansion over this range is considered abnormal. An unstable pre-mutation allele consists of more than 55 CGG repeats which results in increased levels of the mRNA in order to keep the normal level of the FMRP protein. This may be due to a compensatory mechanism derived from a translation problem of the premutated mRNA (18). A new syndrome has been described in males and females older than 50-60 years of age, carrying a premutated allele it is known as Fragile X premutation tremor/ataxia syndrome (FXTAS), a neurodegenerative disorder with core features of action tremor and cerebellar gait ataxia. Frequent associated findings include parkinsonism, executive function deficits, neuropathy, and dysautonomia. It is caused by increased levels of FMR1-mRNA leading to neurotoxicity in the brain (19).

The physiological effects of FMRP are still not well understood and the mechanisms that explain the pathogenesis of this syndrome remain unclear. FMRP is a mRNA binding protein (20), which is involved in the translational regulation of a specific set of mRNAs (21). FMR1 is a member of a gene family including FXR1 and FXR2. These three genes have very strong homology, overlapping expression patterns in neuronal cells, and they form homo and heterodimers. These features suggest that the differences between some of their physiological roles may be subtle (22). All three proteins function as mRNA binding proteins and they form complexes with additional proteins to transport target mRNA from the nucleus to the cytoplasm in microtubule-dependent movements that drive the complexes to the neurites in PC12 cells stimulated with nerve growth factor (23).

FMRP is primarily observed in tissues of ectodermic origin and is highly expressed in the mouse adrenal medulla without co-expression of FXR1P and FXR2P, suggesting that FMRP may have a specific function in this tissue (24). The adrenal gland mainly secretes catecholamines (epinephrine, norepinephrine and dopamine) and glucocorticoids (cortisol). These hormones are involved in many essential metabolic functions in the body; in particular, they regulate the hypothalamus-pituitary-adrenal axis (HPA) that allows the organism to adapt to stressful situations (25). Adrenal activity in the postnatal period is essential for normal development of the HPA axis. There is evidence that FraX is associated with alterations in the action of the HPA axis (26, 27). Recently, abnormalities in glucocorticoids secretion in FraX individuals and in the FraX experimental model, *Fmr1*-knockout mice, have been reported (28, 29). The main targets of glucocorticoids in the brain are the hippocampus, amygdala and cortex, with an active role in the adaptive response of the organism to stress processes, and an impact in the learning process and memory (spatial orientation, or declarative and spatial memory) (30, 31). Also, an abnormal catecholamine content has been demonstrated in the *Fmr1*-knockout mouse model (32).

Recent studies indicate that the absence of FMRP changes the expression of many proteins such as those implicated in RhoGTPase signalling (GDI, RhoA), REDOX processes (Superoxide Dismutase, Glutathione peroxidase, SCD1, Pi3 Kinase) and neurotransmission (GabaA receptor or glucocorticoid receptor) (33,34). Our previous results indicate an excess of Rac1GTPase activation leading to NADPH oxidase-dependent activation and high levels of free radical production in the brain of the *Fmr1*-knockout mice. Moreover, an elevated oxidative stress and an alteration in antioxidant systems, including glutathione (GSH) decrease, are also observed in the *Fmr1*-knockout brain (35). It has been demonstrated that oxidative stress increases or accumulates selectively in CA3 and DG of the ventral hippocampus in psychiatric disorders. Such redox dysregulation alters stress and emotion-related behaviours but leaves intact spatial abilities, indicating functional disruption of the

ventral but not dorsal hippocampus. Thus, a GSH deficit affects PV-IR interneuron's integrity and neuronal synchrony in a region- and time-specific manner, leading to behavioural phenotypes related to psychiatric disorders (36).

The central nervous system (CNS) is highly sensitive to oxidative stress due to its specific anatomical and physiological characteristics. Neurons consume oxygen (O_2) and produce ATP to maintain intracellular gradients of different ions (K^+ , Na^+ , Ca^{2+}). Free radicals from oxygen and nitrogen (ROS and RNS) are involved in REDOX regulation of several protein functions such as Glutamate carriers or neurotransmitter receptors, and their increase leads to excitotoxicity processes that affect cellular functions, and provoke cellular death in the long-term (37).

It is well known that REDOX regulation is involved in many important cellular mechanisms in neurons, astrocytes and microglia, such as the activation of MAPK cascade (mitogen activated protein kinase) (ERK/12, JNK1/2, p38MAPK), Ca^{2+} release and the activation of apoptotic processes (38-40). ROS produced by mitochondrial proteins or membrane proteins (like NADPH-oxidase activated by Rac1) have a role in physiological plasticity and may be required for normal cognitive functions (41). An excess of ROS, however, can induce harmful changes in cellular physiology. Cells can be protected from oxidation with antioxidant and detoxification processes, for example through the activation of the glutathione (GSH) system (42).

Glutathione plays a critical role as an antioxidant, enzyme co-factor, the major redox buffer, and as neuromodulator in the central nervous system. Cysteine has itself neurotoxic effects mediated by free radical generation, increasing extracellular glutamate, and triggering over-activation of N-methyl-D-aspartate (NMDA) receptors (43). GSH can also serve as a neuromodulator/neurotransmitter. GSH binds via its gamma-glutamyl moiety to NMDA receptors (44). GSH is thought to exert dual (agonistic/antagonistic) actions on neuronal responses mediated by NMDA receptors in the brain. GSH also serves as an endogenous NO reservoir by forming S-nitrosoglutathione (GSNO) (30). GSNO can release NO under certain conditions with biological effects, whilst GSNO has a protective effect in the brain under oxidative stress conditions (45). In addition, GSH is also required for cell proliferation and neuronal differentiation (46, 47).

GSH deficiency has been implicated in neurodegenerative diseases. GSH is a tripeptide comprised of glutamate, cysteine, and glycine. Cysteine is the rate-limiting substrate for GSH synthesis within neurons. Most neuronal cysteine uptake is mediated by sodium-dependent excitatory amino acid transporter (EAAT) systems, known as excitatory amino acid carrier 1 (EAAC1). Previous studies have demonstrated that EAAT is vulnerable to oxidative stress, leading to impaired functions (48).

Oxidative stress can activate genes that encode the enzymes of antioxidant defence or transcription factors (NF- κ B, AP1, Nrf2 y NF-AT) and many other structural proteins. The increase of Ca^{2+} in neurons can activate other enzymes including Kinase-C protein (PKC), phosphatase, phospholipase, nNOS, and xanthine oxidase (37).

The normalization of oxidative stress can represent a new experimental target to treat disorders caused by an excessive production of free radicals. Oxidative stress has been found in neurological disorders, including epilepsy, Parkinson's disorder, Down syndrome, Rett syndrome, Autism and Alzheimer's disease (49). It has been demonstrated that neuronal damage due to oxidative stress, and/or hyperadrenergic states can be prevented

by treatment with free radical scavengers or specific compounds acting to prevent free radical production. It has also been shown that neuroprotective therapy prevents neuronal damage in neurodegenerative diseases like Parkinson's and Alzheimer's disease (50, 51). Nutrient deficiencies are common in attention-deficit hyperactivity disorder (ADHD). Supplementing the diet with minerals, vitamins, essential fatty acids omega-3 and omega-6, bioflavonoids, and phosphatidylserine improved ADHD symptoms of the disorder (52). Nutritional status is also related to intelligence, the treatment of mothers during pregnancy and lactation with eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA), examples of very-long-chain n-3 fatty acids, enhances the IQ in children (53).

Currently, the pharmacological treatment used for the FraX has limited effects over the observed symptoms in patients. Stimulants of the central nervous system, such as methylphenidate, are used to treat hyperactivity, and antipsychotic drugs, such as Risperidone, are used to treat aggressive behaviour. Several drugs have been used to treat anxiety, such as Alprazolam or Lorazepam. Patients with epilepsy have been prescribed anticonvulsive drugs (54). In general, a drug or drug combinations are used to treat clinical symptoms; however there are no specific drugs to prevent the appearance of the disorder.

Recent experiments in animal models introduce a new hypothesis for a specific treatment of the disorder using the antagonists of glutamate receptors (55, 56). It has been shown that some features in FraX mice can be normalized by the genetic deletion of the metabotropic glutamate receptor 5 (mGluR5) gene (57). Recent studies have identified ROS as downstream signalling molecules of group I mGluRs activation (58). Furthermore, a previous study has also demonstrated that a double knockout of the genes coding for FMRP and the p21-activated kinase proteins prevent the FraX phenotype (59). These new findings are opening a new path for therapeutic research in the Fragile X Syndrome.

Altered glucocorticoid secretion observed in FraX individuals might contribute to the loss of neurons in the hippocampus demonstrated through autopsies. Neuronal loss and the excess of cortisol may be related to hyperactivation of glucocorticoid receptors in the hippocampus and other brain areas such as amygdala and cortex (60). The long lasting activation of glucocorticoid receptors during development is known to affect the proliferation of neuronal precursors and increase the activation of glial cells, such as astrocytes (61). Furthermore, an altered adrenal secretion can produce an imbalance in brain oxidative stress that will lead to lipid and protein oxidation in the cell membranes. These changes alter the correct function of the synapses between neurons, affecting learning and behaviour, and in the long term will lead to intellectual impairment (62, 63).

High-dose vitamin E supplementation may improve insulin action and decrease plasma fasting insulin and glucose levels by decreasing cellular oxidant stress, altering membrane properties, and decreasing inflammatory activity (64). Increased vitamin E intake may enhance the endogenous cellular antioxidant defence system and reduce levels of ROS that are produced by mitochondria. Vitamin E can also act at the cellular level independently of its antioxidant activity and may potentially contribute to improved insulin action through the inhibition of protein kinase C (65); the decrease of intracellular levels of diacylglycerol (66) and the activation of insulin substrate protein-1 (67).

Vitamin E has also been used in children; most of the clinical data available are for α -tocopherol or tocopherol esters, such as α -tocopheryl acetate. Its use is well documented in diseases such as abetalipoproteinemia (68), cystic fibrosis (69–71), β -thalassemia, sickle cell anemia (72), inborn metabolism errors (73), epidermolysis bullosa (74), glucose-6 phosphate

dehydrogenase deficiency (75), and focal segmental glomerulosclerosis (76). Many studies do not state the rationale for dose calculation, and dosing regimens are not evaluated systematically. In children with cystic fibrosis, the doses differed among the studies: 5.5–47.4 IU kg⁻¹ day⁻¹, 5–10 mg kg⁻¹ day⁻¹, and 50–100 IU/day (77, 78).

Vitamin C has also been widely used in sick children; most of the clinical data available are for ascorbic acid or antioxidant combinations. Its use is well documented in diseases such as aphtous stomatitis (79), infant burns (80), Attention Deficit Hyperactivity Disorder (81) and hyperlipidemia and arterioscleroses (82). An oral dosage of 2000 mg/m(2)/day of Ascorbate may modulate the generation of reactive oxygen species and augment neutrophil apoptosis, which could prevent neutrophil-mediated inflammation in children (79). A 12-month high-dose (30 mg/kg/day) trial of oral ascorbic acid was reported to be safe and well tolerated in children (2–16 years) (83). Vitamin C was also administered as it enhances the regeneration of oxidized vitamin E. Kinetic analysis and studies of vitamin E regeneration in a protein-denaturing system revealed that ascorbate regenerates vitamin E by a nonenzymic mechanism, whereas glutathione regenerates vitamin E enzymatically. It was suggested that a significant interaction occurs between water- and lipid-soluble molecules at the membrane-cytosol interface and that vitamin C may function in-vivo to repair the membrane-bound oxidized vitamin E (84, 85).

2. Methods and design

We have designed a clinical trial to evaluate the effects of an antioxidant combination of ascorbic acid and alpha-tocopherol on the clinical condition of patients with FXS. The study includes patients from 6 years up to the age of 18 diagnosed with FXS; this limit was chosen as it is at this age when a decline in hyperactivity and behavioural symptoms may occur. The minimum duration of treatment and follow-up is 6 months. The symptoms most easily measured are the presence and severity of behavioural abnormalities.

We introduce a new therapeutic approach to FXS, based on the hypothesis that an increase in free radical production and a deficit of vitamins are involved in the pathology and this often provokes severe comorbidity. Moreover, we take into account that current treatment protocols are frequently ineffective among young children and present important potential side effects.

Thus, we propose the following:

Main goal – to show that the combination of 10 mg/Kg/day tocopherol and 10 mg/Kg/day ascorbic acid reduces hyperactivity and behaviour abnormalities among patients aged 6–18 years compared to placebo treatment.

Secondary goals – to assess the safety of the treatment in terms of adverse or unexpected events; to describe metabolic changes resulting from the treatment, as revealed by blood measurements; and to measure the impact of this treatment on the quality of family and scholar life.

2.1 Design

2.1.1 Type of clinical trials

Double blind, randomized clinical study, Phase II.

The study began in December 2010 and is currently in progress.

2.1.2 Recruitment of patients

The patients included are those diagnosed with FXS, according to molecular biology test, currently presenting symptoms. Paediatric Neurologists of the healthcare system were informed about the clinical trial in the Andalusian region, so patients could be referred to the sites where the study is being carried out. In order to maintain double blind conditions, the doctors responsible for patient evaluation derived each patient to the pharmacy department to be allocated to one of the two study groups, using a randomization program. Informed consent was obtained from parents or guardians, and none of the exclusion criteria were present. (See Figure 1).

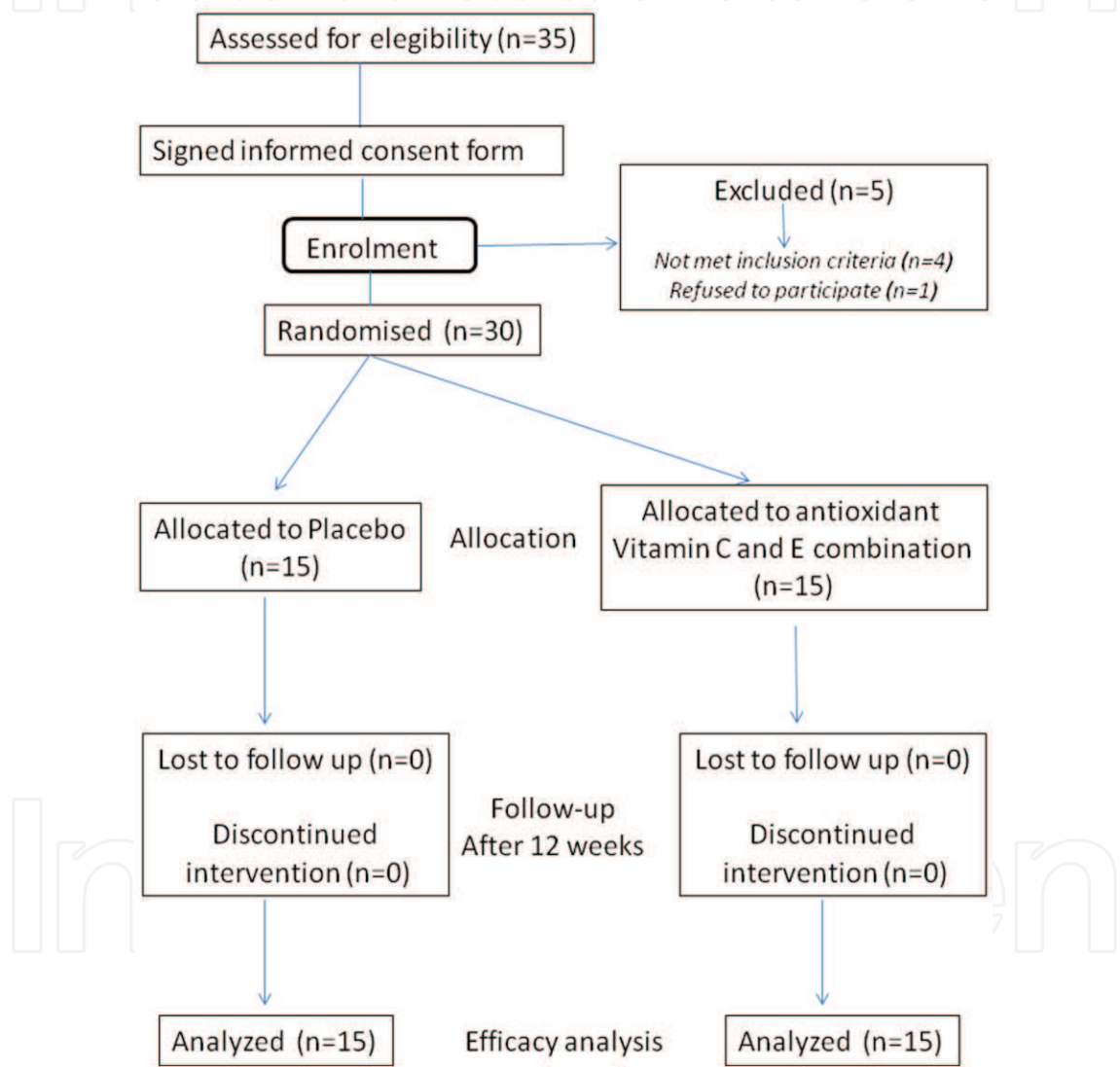


Fig. 1. Trial Flow Chart.

2.2 Study subjects

2.2.1 Patients

Male patients diagnosed with FXS, aged 6–18 years, with clinical and behavioural symptoms of the disorder.

2.3 Selection criteria

2.3.1 Criteria for inclusion

- Male patients aged 6 to 18 years. This is the age range during which the natural course of the illness is most exacerbated. Before the age of 6, hyperactivity may not yet have appeared. After 18 years, behavioural symptoms tend to stabilize.
- Informed consent of the child's parents or guardians, and reasoned agreement with patients older than 12.
- Molecular diagnosis of FXS, according to molecular biology criteria of having more than 200 CCG and hypermethylation of the promoter region of the FMR1 gene.
- Hyperactivity and behavioural symptoms of the disorder.

2.3.2 Criteria for exclusion

- Severe neurological condition not clinically controlled.
- Unrelated neurological disorder.
- Allergy to formula components (including excipients).

2.3.3 Randomization, blinding and assignment to treatment group

- Criteria set out above (age, diagnosis, consent).
- Current pharmacological treatment for behavioural symptoms.
- No contraindication due to the exclusion criteria.

Patients who fulfil these criteria will be included, randomly, in one of the two groups of treatment.

Randomization was centralized and performed after the patient group was studied at T0. A software program was used to ensure that allocation concealment is maintained within the pharmacy department at the hospital. The randomization code will be kept in the pharmacy department responsible for dispensing the corresponding medication. Randomization to either the treatment or the placebo group will only be performed when a patient, suffering FXS, is considered eligible to receive the medication included in this study (See figure 2).

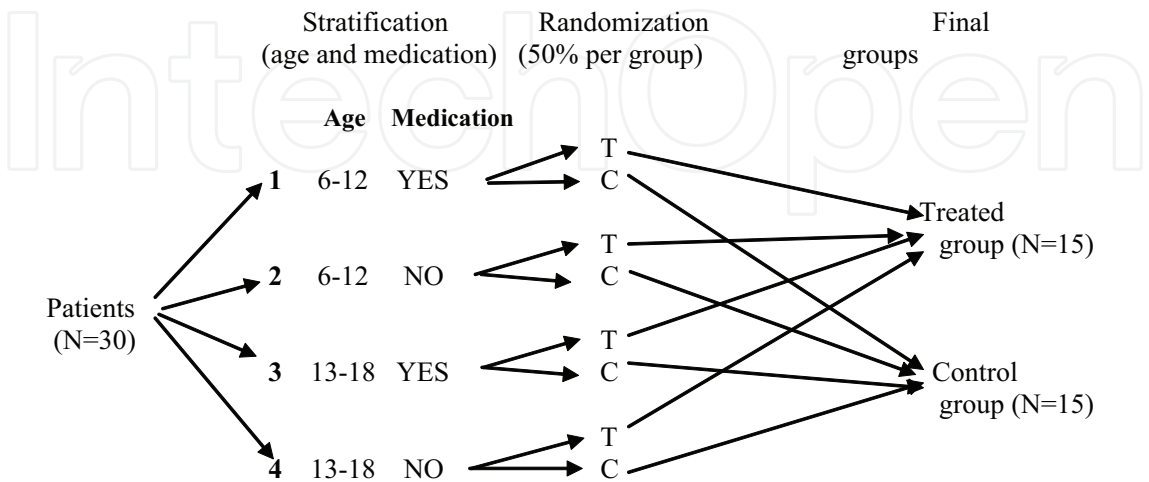


Fig. 2. Randomization Criteria for the Trial. Randomization by blocks and stratification for confusion factors (Age and concomitant medication).

2.3.4 Evaluations

The clinical diagnosis of FXS will be confirmed, and the Conner's score ascertained, so that the patient may be included in the study and any subsequent fall in the global score recorded (at t0, t1).

2.3.5 Withdrawal of individual patients

Patients may withdraw from the study at any time, for any reason and without suffering any sanction for doing so. The researcher-collaborator, after consulting with the principal investigator and the study coordinator, may also interrupt the treatment program if the fact of continuing this treatment, in his/her opinion, is prejudicial to the patients welfare. If a patient withdraws or is withdrawn from the study, follow-up to day 90 shall be continued whenever possible.

2.4 Ethical criteria

2.4.1 Applicable regulations

The study was carried out in accordance with the principles of the Helsinki Declaration, specifically the EMEA/CPMP declaration on the use of the placebo in clinical trials, with respect to the revised Helsinki Declaration, and in accordance with the guidelines for Good Clinical Practice (CPMP/ICH/135/95 – 17 July 1996), as well as local regulations.

2.4.2 Recruitment

The study protocol was approved by the Ethics Committee of the Hospital Carlos Haya (Malaga, Spain). Implementation of the study began after the Spanish national healthcare authorities gave their official approval. Although patients were informed about the freedom of leaving the study at any time, we were interested in the recruitment of those offering the maximum probability of remaining within the study until its conclusion.

2.4.3 Informed consent for minors

After identifying candidate patients for inclusion in the clinical trial, the parents/guardians were provided with all available information and any complementary information that they could require, and they were given an information document so that their informed consent for the children participation in the trial was obtained. The signed form was given to the researcher when the child attended the clinic for the first basal evaluation (t0).

2.4.4 Liability for injury

According to Spanish Law regarding clinical trials, an insurance policy for civil liability was subscribed to cover any injuries that may arise from the performance of the study.

2.5 Treatment details

2.5.1 Dosage and administration of medication

The medication used in the trial was administered orally, at the patients' home. The following medication was provided: Tocopherol acetate, 10 mg/kg/day, was administered

in two daily doses with a maximum of 600 mg/day. Ascorbic acid, 10 mg/kg/day, was administered in two daily doses with a maximum of 800 mg/day.

2.5.2 Preparation and labelling of treatment procedures

The medication for the trials was prepared, labelled and stored by the pharmaceutical service at the "Virgen de las Nieves" Hospital (Granada, Spain). The active principles of the treatment group were obtained via commercially available drugs. The placebo used was created in the hospital's pharmacy department, emulating the excipient and volume of the experimental medication (Colloidal Silica). Procedures for reducing the volume of medication per pack were implemented in accordance with ICH requirements. The study coordinator supervised all procedures applied in this respect.

2.5.3 Other medication allowed

The patients continued taking their usual medication to control symptoms or associated comorbid pathologies. Moreover, they continued receiving any pre-existing psychological or educational therapies. In addition, they continued taking any medication prescribed prior to the recruitment in the study.

2.6 Specific methods

2.6.1 Evaluation of effectiveness

The clinical evaluation of the patients was carried out by applying the Conner's Parent Rating Scale-Revised: long Form [CPRS-R] (86), and the Conner's Teacher Rating Scale-Revised: long Form [CTRS-R], (87).

This scale was designed to study hyperactivity and has been validated for its use with children. The Conner's score was applied by means of a structured questionnaire with multiple informants (generally, parents and teachers) to assess the child's behaviour over a period of at least three months. The translation into Spanish and its adaptation to local conditions were previously validated (88, 89).

2.6.2 Adverse events

Any adverse event notified spontaneously by the subject, or observed by the researcher or by the research team was recorded on the specific form designed for this purpose.

2.6.3 Follow-up after occurrence of an adverse event

All adverse events were observed until their remission or stabilization. Depending on the circumstances, this observation might necessitate evaluation by and/or referral to the patients' GP or to a specialist.

2.7 Procedures and control

2.7.1 Selection of subjects

Patients diagnosed with FXS were included in a preliminary "potential subjects" group. Before undertaking any selection activity, written informed consent, signed and dated,

was obtained from the parents or guardians. Patients' parents were informed, before any action was taken, of the purposes of the study, and any doubts expressed were answered. It was highlighted that they have the unconditional right to withdraw from the study at any time.

2.8 Data analysis

2.8.1 Calculation of the statistical power; establishing the sample size; safety

The sample size was established by means of a pilot scheme based on a phase II effectiveness trial, with 30 patients monitored over 3 months, for a level of significance of 0.05 and a statistical power of 0.8, taking the least favourable case. On the basis of the prevalence of the FXS, 13 patients per group (26 in total) were needed. This sample size was then over-dimensioned to allow for a possible dropout rate of 10%, and so the minimum sample size was calculated to be $n = 30$ (15 patients per group).

3. Results

We have previously shown that NADPH-oxidase is highly activated in brain from Fmr1-knockout mice compared to wild type (35). It is implicated in the production of free radicals, acting as a relevant source of ROS in brain tissue. Furthermore, we have also demonstrated that chronic treatment with antioxidants was able to reduce the behavioural and learning hallmarks in the Fmr1-Knockout mouse (32, 90). In order to understand if an antioxidant combination of Ascorbic acid and Alpha-tocopherol, two well known antioxidants, is useful to reduce Fragile X patient's symptoms we performed a pilot clinical trial in 30 patients affected with the Fragile X syndrome.

Compared to the placebo group, those individuals receiving the antioxidant supplement showed an improvement in behaviour functioning measured by the Parent Conner's Rating scales. Pill counts indicated good compliance with the regimen, and no serious adverse events attributed to the treatment were noted.

The demographic characteristics of the study population are presented in Table I. The average age of the participants was 11,6 (SD 4,2) years, 12.1 (SD 3.4) in the treated group and 11.7(SD 4.8) in placebo group. Based upon a review of psychological testing records, 80% of the controls and 75% of the treatment group were in the hyperactivity range according to the DSMIV criteria. In the placebo group, 45% were in the severe to profound range of hyperactivity, whereas 35% of the treatment group was in this category; a difference which was not statistically significant at the 0.05 level. Figure 1 displays a flow diagram that describes the participation from screening to the conclusion. Thirty participants initiated the trial, 15 of them taking antioxidant supplements and 15 taking a placebo. 100% of participants in both groups remained in the study at the 12 week study visit (t1).

In the treatment group, among those participants with associated seizure disorder, 2 out of 15 participants had at least one seizure prior to enrolling in the study and were taking anticonvulsant drugs. While in the placebo group none of the participants had a seizure before entering the study. Asthma, obsessive compulsive disorder and autistic features were present in the patients included in the trial (see table 1).

Patient Characteristics	Active (<i>n</i> = 15)	Placebo (<i>n</i> = 15)	Total (<i>n</i> = 30)
Age, years, M(SD)	12.1(3.4)	11.7(4.8)	11.6(4.2)
Age groups, <i>n</i> (%)			
6–12 years	7(56)	8(44)	15 (50)
13–18 years	8(37)	7(63)	15(50)
Gender, <i>n</i> (%)			
Male	15(50)	15(50)	30(100)
Weight M(SD)	53.2(6.6)	50.9(6.6)	52.1(4.6)
Psycopharmacological treatment, <i>n</i> (%)	11(36,66)	11(36,66)	18 (60)
Parent Conner’s Rating scales, M(SD)			
DSM-IV Hyperactive/impulsive	67.8(12.1)	64.2(12.0)	66.0(12.0)
DSM-IV Inattentive	60.3(8.8)	62.2(9.3)	61.3(9.0)
Teacher Conner’s Rating scales, M(SD)			
DSM-IV Hyperactive/impulsive	62.8(10.3)	64.7(14.4)	63.7(12.3)
DSM-IV Inattentive	64.6(6.7)	67.6(8.1)	66.1(7.5)
Associated conditions, <i>n</i> (%)			
Epilepsy	2(6.6)	0(0)	2(6.6)
Asthma	2(6.6)	0(0)	2(6.6)
Autistic traits	2(6.6)	2(6.6)	4(13.2)
Obsessive compulsive disorder	0(0)	1(3.3)	1(3.3)

Note: DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; M =Mean; SD =Standard deviation; *n* =number of patients; *p* = statistic significance; % = percentage.

Table 1. Demographics of Sample

According to the results of the Parent Conner’s Rating scales for the DSM-IV Hyperactive/impulsive subscale, this symptom was present in 22 participants. 70% (7 out of 10) in the treated group and 16,6% (2 out of 12) in the placebo group significantly reduced this symptom after 12 weeks of antioxidant treatment (*p*<0.05). The reduction was mainly observed in the younger group of patients, 87,7 % significantly reduced hyperactive behaviour of those between 6 and 12 year old in the treated group (See table 2).

Patient Characteristics	<i>n</i> (Baseline)	Active	Placebo	<i>p</i>
Total	30	(11/15)	(4/15)	<0.05
Age groups				
6–12 years	15	(7/8)	(1/8)	<0.05
13–18 years	15	(4/7)	(3/7)	ns
Parent Conner’s Rating scales M(SD)				
DSM-IV Hyperactive/impulsive	22	(7/10)	(2/12)	<0.05
DSM-IV Inattentive	21	(5/9)	(4/12)	ns
Teacher Conner’s Rating scales, M(SD)				
DSM-IV Hyperactive/impulsive	23	(5/12)	(2/11)	ns
DSM-IV Inattentive	26	(8/13)	(2/13)	ns

Note: DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; M =Mean; SD =Standard deviation; *n* =number of patients; *p* = statistic significance; % = percentage.

Table 2. Patient Characteristics and Response Rates in Subgroups

4. Discussion

FXS is considered to be a rare neurodevelopmental disease, although different rates of prevalence are being reported in current studies (2, 5). The condition is seldom diagnosed in Spain, due to unawareness of its existence and characteristics (5). Until very recently, FXS was only recognized as such for the most severe cases, in which there was an important degree of functional limitation or very evident autism. Few clinical trials have been carried out with children affected by FXS, probably due to its consideration of a rare disease, in addition to the normal difficulties in this kind of study and the special ethical and legal considerations to protect minors. Nevertheless, such studies are clearly needed (33).

FMRP is involved in the regulation of proteins causing brain oxidative stress, so in the absence of FMRP there is hyperactivation of RAC1-GTPase dependent NADPH-oxidase signalling. These alterations lead to an excess of free radical production and then, when antioxidants are unable to counteract the production of free radicals, this fact at in the long term produces oxidative stress which is a crucial factor in the central nervous system that disrupts neuronal, astrocyte and microglia communication (36). Evidence of oxidative stress in FXS is manifested through high levels of oxidised proteins, lipid peroxidation end products, formation of protein-carbonyls and oxidative alteration of the glutathione system in the brain of the *Fmr1*-Knockout mouse model (32, 35, 90).

Since 1983, it has been indicated that vitamins can improve Fragile X patients' symptoms. The first vitamin used for the treatment of the FXS was folic acid, and several publications assessed its efficacy and safety (91-96).

Two double-blind trials have assessed the safety and efficacy of L-Acetyl-Carnitine (LAC) in boys with FXS and an additional diagnosis of ADHD. Both of these trials were randomized placebo-controlled and used a parallel design. They also reported no significant side-effects in the LAC group (97,98).

There are also enhanced, abnormal epileptic discharges consistent with an enhanced rate of clinical seizures in FXS patients and also auditory-dependent seizures in the mouse model. There are several studies regarding the use of tocopherol to control seizures in animal models and humans (99)

A 4-week, randomized, double blind, placebo-controlled, crossover design was conducted, and either 3 mg/day melatonin or placebo was given to participants for 2 weeks and then alternated for another 2 weeks. The results of this study support the efficacy and tolerability of melatonin treatment for sleep problems in children with FXS (100). Melatonin is known to have antioxidant properties that can be involved in the effectiveness of this treatment.

To assess antioxidant positive effects versus placebo, a one-way crossover study was selected due to the impossibility of abolishing a 'carry-over' of treatment effect from the first period of treatment to the next. A carry-over effect means that the observed difference between the treatments depends upon the order in which they were received; hence the estimated overall treatment effect will be affected (usually underestimated, leading to a bias towards the null) (101).

Orally-administered antioxidants such as Tocopherol and ascorbic acid have been used as a nutritional supplement, and are considered safe even for children. Tocopherol is contraindicated in cases of vitamin K deficiency caused by malabsorption or anticoagulant

therapy. The FDA's recommended daily dose is 10 mg/day, but the tolerable upper intake level is considered 300 mg/day (102). In our trial, we decided to use a maximum dose of 600 mg/day as it was proven in many other previous studies to be safe and give a therapeutic dose (85).

Vitamin E (alpha-tocopherol) is a liposoluble vitamin with a wide therapeutic margin. In clinical and pharmacological trials, it has been shown to have interesting properties, participating in oxidative deamination, transamination and decarboxylation; it also participates in the decarboxylation of glutamic acid to GABA, from DOPA to dopamine and from 5-hydroxytryptophan to serotonin. It presents anti-convulsant properties and seems to exercise a neuroprotective and antitoxic effect. It can be administered to children, and has been authorized for use to treat children with alterations in character, language and behaviour; learning difficulties; delayed learning to walk; convulsive illnesses; intoxication of the central nervous system; trembling; and Parkinson's disease. The dosage provided may vary widely, as renal elimination ensures its toxicity is minimal (101).

The follow-up period of three months is based on previous trials and also considered a minimum period to improve symptoms such as behaviour and antioxidant status. We believe that if the patient enters the analysis with a Conner's T-score higher than 55, it will be easier to identify significant differences, with the symptoms being controlled to a greater extent, and more quickly, among the experimental group than among the control group.

The combined application of these measurement methods, namely the objectification of FXS behavioural symptoms will enable us to reach an objective judgment of the effectiveness and safety of the treatment being tested.

In summary, treatment for FXS continues to present important shortcomings and further clinical trials are necessary in this respect, especially among children showing more severe symptoms. Our result demonstrates, for the first time, the efficacy of antioxidant combination to control behaviour in the fragile X patients showing moderate or severe hyperactivity.

5. Abbreviations

ADHD: Attention deficit/hyperactivity disorder; CONSORT: Consolidated Standards of Reporting Trials; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders; FDA: Food and drugs administration; GABA: Gamma aminobutyric acid; MPA: Monophosphate adenosine; NMDA: N-methyl-D-aspartate; POV: Principal outcome variable; SAE: Severe adverse event; CPRS-R: Conner's Parent Rating Scale-Revised: long Form. CTRS-R. Conner's Teacher Rating scale-revised. CBC: Child Behaviour Checklist.

6. Competing interests

The authors declare that they have no competing interests.

7. Authors' contributions

LPC, RCM, IFC, CQN, MTLF: Clinical review of the subject and previous consideration, development of the phase II study. LPC, YDO, IAH: Methodological design. YDO and LPC:

Preparation of documentation. YDO: Preparation of the AEMPS and Andalusian Clinical trial committee permits and also International Registry entry of the clinical trial. RCM and CQN: Training with and standardization of procedures and clinical measurement instrumentation. LPC: Review and decision making standpoint on medication.

8. Acknowledgements

This protocol is approved and funded by the Spanish Ministry of Health, Research Fund (TRA152 and EC191), the Spanish Innovation and Science Ministry (SXF2008-00486). The Health Ministry of the Andalusian Regional Government (PI09-0507), the Economic, Innovation and Science Ministry of the Andalusian Regional Government (P10-CTS-05704), and Jerome Lejeune Foundation (Paris, France). We thank the patients and their families for participation. The authors wish to thank the following for their support: Fragile X Syndrome Association in Andalusia; Fragile X syndrome Association in Madrid; Fragile X syndrome Association in Extremadura; the Spanish Federation of Fragile X syndrome; Rare disorder Spanish Federation (FEDER); the Carlos Haya Hospital (part of the Andalusian Public Health Service). We thank D. W. E. Ramsden for the revision of the paper.

9. References

- [1] Martin JP, Bell J. A pedigree of mental defect showing sex-linkage. *J Neurol Psych* 1943; 6:154-157.
- [2] Sutherland GR and Ashford PLC. X-linked mental retardation with macro-orchidism and the fragile site at Xq27 or 28. *Hum Genet* 1978; 48:117-120.
- [3] Chakrabarti L, Davies KE. Fragile X syndrome. *Curr Opin Neurol*. 1997; 10(2):142-147.
- [4] Sherman SL. Genetic epidemiology of the fragile X syndrome with special reference to genetic counseling. *Prog Clin Biol Res* 1991; 368:79-99.
- [5] Fernandez-Carvajal I, Walichiewicz P, Xiaosen X, et al. Screening for expanded alleles of the FMR1 gene in blood spots from newborn males in a Spanish population. *J Mol Diagn* 2009; 11(4):324-329.
- [6] Rife M, Badenas C, Mallolas J, et al. Incidence of fragile X in 5,000 consecutive newborn males. *Genet Test* 2003; 7(4):339-343.
- [7] De Vries BB, Mohkamsing S, van den Ouweland AM, et al. Screening and diagnosis for the fragile X syndrome among the mentally retarded: an epidemiological and psychological survey. Collaborative Fragile X Study Group. *Am J Hum Genet* 1997; 61, 660-667.
- [8] Hagerman RJ. Fragile X Syndrome: Diagnosis, Treatment, and Research. Johns Hopkins University; New York 2002; 3-109.
- [9] Siomi H, Matunis MJ, Michael WM, et al. The pre-mRNA binding K protein contains a novel evolutionarily conserved motif. *Nucleic Acids Res* 1993; 21, 1193-1198.
- [10] Bakker C E, Verheij C, Willemsen R et al. Fmr1 knockout mice: A model to study fragile X mental retardation. *Cell* 1994; 78, 23-33.
- [11] Chen L, Toth M. Fragile X mice develop sensory hyperreactivity to auditory stimuli. *Neuroscience* 2001; 103(4):1043-1050.

- [12] Bakker CE, de Diego Otero Y, Bontekoe C, et al. Immunocytochemical and biochemical characterization of FMRP, FXR1P, and FXR2P in the mouse. *Exp Cell Res* 2000; 258(1):162-170.
- [13] Musumeci SA, Bosco P, Calabrese G, et al. Audiogenic seizures susceptibility in transgenic mice with fragile X syndrome. *Epilepsia* 2000; 41(1):19-23.
- [14] Schapiro MB, Murphy DG, Hagerman RJ, et al. Adult fragile X syndrome: neuropsychology, brain anatomy, and metabolism. *Am J Med Genet* 1995; 60(6):480-493.
- [15] Lubs H. A marker X chromosome. *Am J Hum Genet* 1969; 21:231-244.
- [16] Verkerk AJMH, Pieretti M, Sutcliffe JS, et al.: Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991; 65:905-914.
- [17] Fu YH, Kuhl DP, Pizzuti A, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the Sherman paradox. *Cell* 1991; 67:1047-1058.
- [18] Hessler D, Tassone F, Loesch DZ, et al. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. *Am J Med Genet B Neuropsychiatr Genet* 2005; 139(1):115-121.
- [19] Jacquemont S, Hagerman RJ, Leehey M, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet* 2003; 72:869-878.
- [20] Lai D, Sakkas D, Huang Y et al. The fragile X mental retardation protein interacts with a distinct mRNA nuclear export factor NXF2. *RNA* 2006; 12(8):1446-9.
- [21] D'Hulst C, De Geest N, Reeve SP, et al. Decreased expression of the GABA(A) receptor in fragile X syndrome. *Brain Res* 2006; 1121(1):238-245.
- [22] Duan R and Jin P. Identification of messenger RNAs and microRNAs associated with fragile X mental retardation protein. *Meth Mol Biol* 2006; 342:267-276.
- [23] de Diego Otero Y., Severijnen LA, van Cappellen G et al. Microtubule-mediated transport of FMR1-protein via granules in neurites of PC12 cells. *Mol Cell Biol* 2002; 22(23):8332-8341.
- [24] De Diego Otero Y, Bakker CE, Prawien R, et al. Immunocytochemical characterization of Fmrp, Fxr1p and Fxr2p during embryonic development in the mouse. *Gene function and disease* 2000; 1: 28-37.
- [25] De Kloet ER, Vreugdenhil E, Oitzl MS, et al. Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 1998; 19(3):269-301.
- [26] Hall S, DeBernardis M, and Reiss A. Social escape behaviours in children with fragile x syndrome. *J Autism Dev Disord* 2006; 36(7):935-947.
- [27] Hessler D, Glaser B, Dyer-Friedman J, et al. Social behaviour and cortisol reactivity in children with fragile X syndrome. *J Child Psychol Psychiatry* 2006; 47(6):602-10.
- [28] Hessler D, Rivera SM, Reiss AL. The neuroanatomy and neuroendocrinology of fragile X syndrome. *Ment Retard Dev Disabil Res Rev* 2004; 10(1):17-24.
- [29] Markham JA, Beckel-Mitchener AC, Estrada CM, et al. Corticosterone response to acute stress in a mouse model of Fragile X syndrome. *Psychoneuroendocrinology* 2006; 31(6):781-785.
- [30] Oei NY, Everaerd WT, Elzinga BM, et al. Psychosocial stress impairs working memory at high loads: An association with cortisol levels and memory retrieval. *Stress* 2006; 9(3):133-141.

- [31] Brunner R, Schaefer D, Hess K, et al. Effect of high-dose cortisol on memory functions. *Ann N Y Acad Sci* 2006; 1071:434-437.
- [32] de Diego-Otero Y, Romero-Zerbo Y, el Bekay R, et al. Alpha-tocopherol protects against oxidative stress in the fragile X knockout mouse: an experimental therapeutic approach for the Fmr1 deficiency. *Neuropsychopharmacology* 2009; 34(4):1011-1026.
- [33] D'Agata V, Warren ST, Zhao W, et al. Gene expression profiles in a transgenic animal model of fragile X syndrome. *Neurobiol Dis* 2002; 10(3): 211-218.
- [34] Darnell JC, Jensen KB, Jin P, et al. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 2001; 107(4):489-499.
- [35] el Bekay R, Romero-Zerbo Y, Decara J, et al. Enhanced markers of oxidative stress, altered antioxidants and NADPH-oxidase activation in brains from Fragile X mental retardation 1-deficient mice, a pathological model for Fragile X syndrome. *Eur J Neurosci* 2007; 26(11):3169-3180.
- [36] Steullet P, Cabungcal JH, Kulak A, et al. Redox dysregulation affects the ventral but not dorsal hippocampus: impairment of parvalbumin neurons, gamma oscillations, and related behaviours. *J Neurosci* 2010; 30(7):2547-2558.
- [37] Beal M F. Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Ann Neurol* 1992; 31:119-130.
- [38] Marshall CJ. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* 1995; 80:179-185
- [39] Kurosinski P and Gotz J. Glial cells under physiologic and pathologic conditions. *Arch Neurol* 2002; 59:1524-1628.
- [40] Rosen LB, Ginty DD, Weber MJ, et al. Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. *Neuron* 1994; 12: 1207-1221.
- [41] Kishida KT and Klann E. Sources and targets of reactive oxygen species in synaptic plasticity and memory. *Antioxid Redox Signal* 2007; 9(2):233-244.
- [42] Bazan NG, Marcheselli VL and Cole-Edwards K.. Brain response to injury and neurodegeneration: endogenous neuroprotective signaling. *Ann N Y Acad Sci* 2005; 1053:137-147.
- [43] Janaky R, Varga V, Hermann A, et al. Mechanisms of L-cysteine neurotoxicity. *Neurochem Res* 2000; 25:1397-1405.
- [44] Janaky R, Ogita K, Pasqualotto BA, et al. Glutathione and signal transduction in the mammalian CNS. *J Neurochem* 1999; 73:889-902.
- [45] Rauhala P, Lin AM and Chiueh CC. Neuroprotection by S-nitrosoglutathione of brain dopamine neurons from oxidative stress. *FASEB J* 1998; 12:165-173.
- [46] Poot M, Teubert H, Rabinovitch PS, et al. De novo synthesis of glutathione is required for both entry into and progression through the cell cycle. *J Cell Physiol* 1995; 163: 555-560.
- [47] Sagara J and Makino N. Glutathione induces neuronal differentiation in rat bone marrow stromal cells. *Neurochem Res* 2008; 33: 16-21.
- [48] Aoyama K, Watabe M and Nakaki T. Regulation of neuronal glutathione synthesis. *J Pharmacol Sci* 2008; 108(3):227-238.
- [49] Sultana R, Perluigi M and Butterfield DA. Protein oxidation and lipid peroxidation in brain of subjects with Alzheimer's disease: insights into mechanism of

- neurodegeneration from redox proteomics. *Antioxid Redox Signal* 2006; 8(11-12): 2021-2037.
- [50] Sano M, Ernesto C, Thomas RG, et al. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N Engl J Med* 1997; 336(17):1216-1222.
- [51] Di Matteo V and Esposito E. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Curr Drug Target CNS Neurol Disord* 2003; 2:95-107.
- [52] Riga S and Riga D. An antistress and antiaging neurometabolic therapy. Accelerated lipofuscinolysis and stimulated anabolic regeneration by the antagonic-stress synergistic formula. *Ann N Y Acad Sci* 1995; 771:535-550.
- [53] Helland IB, Smith L, Saarem K, et al. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics* 2003; 111(1):e39-44.
- [54] Berry-Kravis E and Potanos K. Psychopharmacology in fragile X syndrome--present and future. *Ment Retard Dev Disabil Res Rev* 2004; 10(1):42-48.
- [55] McBride SM, Choi CH, Wang Y, et al. Pharmacological rescue of synaptic plasticity, courtship behaviour, and mushroom body defects in a *Drosophila* model of fragile X syndrome. *Neuron* 2005; 45(5):753-764.
- [56] Yan QJ, Rammal M, Tranfaglia M, et al. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology* 2005; 49(7):1053-1066.
- [57] Dölen G, Osterweil E, Rao BS, et al. Correction of Fragile X Syndrome in Mice. *Neuron* 2007; 56(6):955-962.
- [58] Ji G and Neugebauer V. Reactive oxygen species are involved in group I mGluR-mediated facilitation of nociceptive processing in amygdala neurons. *J Neurophysiol.* 2010; 104(1):218-229.
- [59] Lupien SJ, Nair NP, Brière S, et al. Increased cortisol levels and impaired cognition in human aging: implication for depression and dementia in later life. *Rev Neurosci* 1999; 10(2):117-139.
- [60] Madrigal JL, García-Bueno B, Caso JR, et al. Stress-induced oxidative changes in brain. *CNS Neurol Disord Drug Targets* 2006; 5(5):561-8.
- [61] Hessel D, Glaser B, Dyer-Friedman J, et al. Social behaviour and cortisol reactivity in children with fragile X syndrome. *J Child Psychol Psychiatry* 2006; 47:602-610.
- [62] Wisbeck JM, Huffman LC, Freund L, et al. Cortisol and social stressors in children with fragile X: a pilot study. *J Dev Behav Pediatr* 2000; 21:278-282.
- [63] Hayashi ML, Rao BS, Seo JS, et al. Inhibition of p21-activated kinase rescues symptoms of fragile X syndrome in mice. *Proc Natl Acad Sci U S A* 2007; 104(27):11489-11494.
- [64] Bradford A, Atkinson J, Fuller N, et al. The effect of vitamin E on the structure of membrane lipid assemblies. *J Lipid Res* 2003; 44:1940-1945.
- [65] Azzi A, Ricciarelli R and Zingg JM. Nonantioxidant molecular functions of alpha-tocopherol (vitamin E). *FEBS Lett* 2002; 519: 8-10.
- [66] Azzi A, Breyer I, Feher M, et al. Specific cellular responses to alpha-tocopherol. *J Nutr* 2000; 130: 1649-1652.

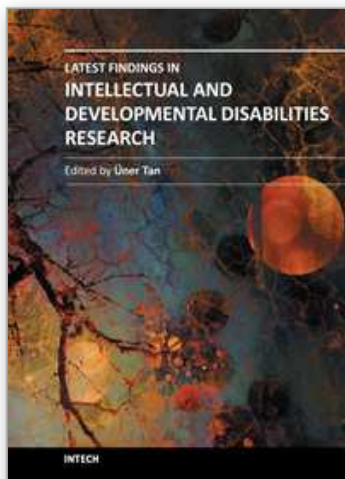
- [67] Griffin ME, Marcucci MJ, Cline GW, et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C and alterations in the insulin signaling cascade. *Diabetes* 1999; 48: 1270–1274.
- [68] Taketomo CK, Hodding JH and Kraus DM. *Pediatric dosage handbook*, 14th ed. Lexi-Comp, Hudson, OH, 2007 pp 1620–1622.
- [69] Feranchak AP, Sontag MK, Wagener JS, et al. Prospective, long-term study of fat-soluble vitamin status in children with cystic fibrosis identified by newborn screen. *J Pediatr*. 1999; 135:601–610.
- [70] Winklhofer-Roob BM, van't Hof MA and Shmerling DH. Long-term oral vitamin E supplementation in cystic fibrosis patients: RRR- α -tocopherol compared with all-rac- α -tocopheryl acetate preparations. *Am J Clin Nutr* 1996; 63:722–728.
- [71] Wilfond BS, Farrell PM, Laxova A, et al. Severe hemolytic anemia associated with vitamin E deficiency in infants with cystic fibrosis. *Clin Pediatr (Phila)* 1994; 33:2–7.
- [72] Brigelius-Flohé R, Kelly FJ, Salonen JT, et al. The European perspective on vitamin E: current knowledge and future research. *Am J Clin Nutr* 2002; 76:703–716.
- [73] Moyano D, Vilaseca MA, Pineda M, et al. Tocopherol in inborn errors of intermediary metabolism. *Clin Chim Acta* 1997; 263:147–155.
- [74] Shirakata Y, Shiraishi S, Sayama K, et al. High-dose tocopherol acetate therapy in epidermolysis bullosa siblings of the Cockayne-Touraine type. *J Dermatol* 1993; 20:723–725.
- [75] Eldamhoughy S, Elhelw Z, Yamamah G, et al. The vitamin E status among glucose-6 phosphate dehydrogenase deficient patients and effectiveness of oral vitamin E. *Int J Vitam Nutr Res* 1988; 58:184–188.
- [76] Tahzib M, Frank R, Gauthier B, et al. Vitamin E treatment of focal segmental glomerulosclerosis: results of an open-label study. *Pediatr Nephrol* 1999; 13:649–652.
- [77] Lavine JE. Vitamin E treatment of nonalcoholic steatohepatitis in children: a pilot study. *J Pediatr* 2000; 136:734–738.
- [78] Vajro P, Mandato C, Franzese A, et al. Vitamin E treatment in pediatric obesity-related liver disease: a randomized study. *J Pediatr Gastroenterol Nutr* 2004; 38:48–55.
- [79] Yasui K, Kurata T, Yashiro M, et al. The effect of ascorbate on minor recurrent aphthous stomatitis. *Acta Paediatr* 2010; 99(3):442–445.
- [80] Barbosa E, Faintuch J, Machado Moreira EA, et al. Supplementation of vitamin E, vitamin C, and zinc attenuates oxidative stress in burned children: a randomized, double-blind, placebo-controlled pilot study. *J Burn Care Res* 2009; 30(5):859–866.
- [81] Joshi K, Lad S, Kale M, et al. Supplementation with flax oil and vitamin C improves the outcome of Attention Deficit Hyperactivity Disorder (ADHD). *Prostaglandins Leukot Essent Fatty Acids* 2006; 74(1):17–21.
- [82] Engler MM, Engler MB, Malloy MJ, et al. Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. *Circulation* 2003; 108(9):1059–1063.
- [83] Burns J, Ouvrier RA, Yiu EM, et al. Ascorbic acid for Charcot-Marie-Tooth disease type 1A in children: a randomised, double-blind, placebo-controlled, safety and efficacy trial. *Lancet Neurol* 2009; 8(6):537–544.
- [84] Chan AC. Partners in defense, vitamin E and vitamin C. *Can J Physiol Pharmacol* 1993; 1:25–31.

- [85] Lott IT, Doran E, Nguyen VQ, et al. Down syndrome and dementia: A randomized, controlled trial of antioxidant supplementation. *Am J Med Genet A* 2011; 155(8):1939-1948.
- [86] Conners CK, Sitarenios G, Parker JDA, et al. The Revised Conners' Parent Rating Scale (CPRS-R): Factor structure, reliability, and criterion validity. *Journal of Abnormal Child Psychology* 1998a; 26: 257-268.
- [87] Conners CK, Sitarenios G, Parker JDA, et al. Revision and restandardization of the Conners Teacher Rating Scale (CTRS-R): Factor structure, reliability, and criterion validity. *Journal of Abnormal Child Psychology* 1998b; 26: 279-291.
- [88] Farré-Riba A, Narbona J. Conners' rating scales in the assessment of attention deficit disorder with hyperactivity (ADHD). A new validation and factor analysis in Spanish children. *Rev Neurol* 1997; 25(138):200-204.
- [89] Achenbach TM, and Edelbrock C. Manual for the child behaviour checklist and revised child behaviour profile. Burlington, VT: 1983. Queen City Printers.
- [90] Romero-Zerbo Y, Decara J, el Bekay R, et al. Protective effects of melatonin against oxidative stress in Fmr1 knockout mice: a therapeutic research model for the fragile X syndrome. *J Pineal Res* 2009; 46(2):224-234.
- [91] Brown WT, Jenkins EC, Friedman E, et al. Folic acid therapy in the fragile X syndrome. *Am J Med Genet* 1984; 17:289-297.
- [92] Carpenter NJ, Barber DH, Jones M, et al. Controlled six-month study of oral folic acid therapy in boys with fragile X-linked mental retardation. *Am J Med Genet* 1983; 35:82A.
- [93] Fisch GS, Cohen IL, Gross AC et al. Folic acid treatment of fragile X males: a further study. *Am J Med Genet* 1988; 30:393-399.
- [94] Hagerman RJ, Jackson AW, Levitas A, et al. Oral folic acid versus placebo in the treatment of males with the fragile X syndrome. *Am J Med Genet* 1986; 23:241-262.
- [95] Madison LS, Wells TE, Fristo TE, et al. A controlled study of folic acid treatment in three fragile X syndrome males. *J Dev Behav Pediatr* 1986; 7:253-256.
- [96] Strom CM, Brusca RM and Pizzi WJ. Double-blind, placebo-controlled crossover study of folinic acid (Leucovorin) for the treatment of fragile X syndrome. *Am J Med Genet* 1992; 44:676-682.
- [97] Torrioli MG, Vernacotola S, Mariotti P, et al. Double-blind, placebo-controlled study of L-acetylcarnitine for the treatment of hyperactive behaviour in fragile X syndrome. *Am J Med Genet* 1999; 87:366-368.
- [98] Torrioli MG, Vernacotola S, Peruzzi L, et al. A double-blind, parallel, multicenter comparison of L-acetylcarnitine with placebo on the attention deficit hyperactivity disorder in fragile X syndrome boys. *Am J Med Genet A* 2008; 146:803-812.
- [99] Levy SL, Burnham WM, Bishai A, et al. The anticonvulsant effects of vitamin E: a further evaluation. *Can J Neurol Sci* 1992; 19(2):201-203.
- [100] Wirojanan J, Jacquemont S, Diaz R, et al. The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *J Clin Sleep Med* 2009 ; 5(2):145-150.
- [101] Kappus H and Diplock AT. Tolerance and safety of vitamin E: a toxicological position report. *Free Radic Biol Med* 1992; 13(1):55-74.

- [102] Ogunmekan AO and Hwang PA. A randomized, double-blind, placebo-controlled, clinical trial of D-alpha-tocopheryl acetate (vitamin E), as add-on therapy, for epilepsy in children. *Epilepsia* 1989; 30(1):84-89.

IntechOpen

IntechOpen



Latest Findings in Intellectual and Developmental Disabilities Research

Edited by Prof. Uner Tan

ISBN 978-953-307-865-6

Hard cover, 404 pages

Publisher InTech

Published online 15, February, 2012

Published in print edition February, 2012

Intellectual and Developmental Disabilities presents reports on a wide range of areas in the field of neurological and intellectual disability, including habitual human quadrupedal locomotion with associated cognitive disabilities, Fragile X syndrome, autism spectrum disorders, Down syndrome, and intellectual developmental disability among children in an African setting. Studies are presented from researchers around the world, looking at aspects as wide-ranging as the genetics behind the conditions to new and innovative therapeutic approaches.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Y. de Diego Otero, C. Quintero-Navarro, Rocio Calvo-Medina, R. Heredia-Farfan, L. Sanchez-Salido, E. Lima-Cabello, A. Higuero-Tapiador, I. del Arco-Herrera, I. Fernandez-Carvajal, T. Ferrando-Lucas and L. Perez-Costillas (2012). Oral Ascorbic Acid and Alpha-Tocopherol to Reduce Behavioural Problems in Young Patients Affected of Fragile X Syndrome: A Randomized, Double-Blind, Placebo-Controlled Phase II Pilot Trial, Latest Findings in Intellectual and Developmental Disabilities Research, Prof. Uner Tan (Ed.), ISBN: 978-953-307-865-6, InTech, Available from: <http://www.intechopen.com/books/latest-findings-in-intellectual-and-developmental-disabilities-research/oral-ascorbic-acid-and-alpha-tocopherol-to-reduce-behavioural-problems-in-young-patients-affected-of>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen