Efficacy of ethyl-EPA as a treatment for Huntington disease: A systematic review and meta-analysis.

Running title: Ethyl-EPA as a treatment for Huntington disease

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Abstract

Objective

After MRI studies suggested theefficacy of ethyl-EPA in reducing the progressive brain atrophy in Huntington disease, trials were conducted to test its efficacy as a treatment for Huntington disease. Trials continued for six months did not find any significant improvement urging discontinuation of the drug. However, trials continued for 12 months indicated improvement of motor functions in these patients.

Methods

We searched 12 electronic databases to find randomized clinical trials relevant to our inclusion criteria. After screening, only five papers were included. Continuous and binary variables were analyzed to compute the pooled mean difference (MD) and risk ratio (RR), respectively. Quality effect model meta-analysis was used as a post hoc analysis for studies at 12 months.

Findings

Meta-analysis indicated that ethyl-eicosapentaenoic acid has no significant effect on any scale of HD at six months. At 12 months, two studies suggested significant improvements of the total motor score and total motor score -4 in both fixed and quality effect model [MD = -2.720, 95% CI (-4.76; -.68), *P = 0.009*], [MD = -2.225, 95% CI (-3.842; -0.607), *P = 0.007*] respectively. maximal chorea score showed significant results [MD = -1.013, 95% CI (-1.793; -0.233), *P = 0.011*] in only fixed effect model, while no improvement was detected for Stroop color naming test or symbol digit modality.

Conclusion

Meta-analysis indicated a significant improvement of motor scores only after 12 months. These results should be interpreted cautiously because only two studies had

assessed the efficacy of ethyl-EPA after 12 months with one of them had six months open-label phase.

Keywords:

Huntington Disease, Chorea, Eicosapentaenoic Acid, Omega 3 Fatty Acids

Summation

- In this meta-analysis, we found that ethyl-EPA significantly improved motor functions in Huntington disease after 12 months.
- Ethyl-EPA significantly decreased brain atrophy in MRI studies after 6 months and the effect was evident clinically on motor symptoms after 12 months; however, the 12 months' results should be interpreted cautiously as the second six months of TREND-HD study was open label.
- Despite the results of clinical trials after six months, more trials are needed to investigate ethyl-EPA effect after 12 months and test its impact on pathways responsible for brain atrophy.

Consideration

- These results should be taken with caution as only two studies continued for 12 months
- The MRI studies has small sample size of 19 patients in ethy-EPA group versus only 22 in Placebo group.

Introduction

Huntington disease (HD) is one of the nine well-known polyglutamine genetic disorders of the central nervous system (1,2) with a worldwide prevalence of 2.71 per 100.000 (3). It has a higher prevalence in Europe, North America, and Australia (5.7 per 100.000) compared to Asia (0.40 per 100.000) (3). HD is caused by an autosomal dominant inheritance resulting in a high-penetrance genetic mutation in the gene coding for huntingtin protein (4). This mutation causes a repetition of cytosine-adenine-guanine (CAG) sequence that codes for amino acid glutamine (1,4). Therefore, the trinucleotide repeat expansion leads to the production of mutant huntingtin protein causing the neuronal death in the cerebral cortex and basal ganglia (4).

Normally, CAG is repeated from 15 to 27 times, while in HD patients, CAG was found to be repeated 19 -31 times in many patients (5). Furthermore, the age of onset of HD depends mainly on the CAG repeats; in a review by Langbehn et al., it was found that the mean age of onset was indirectly proportional to CAG repeats (5).. The core neurologic symptoms of the disease include three categories: the motor changes, cognitive disabilities, and behavioral manifestations (6,7). The Huntington chorea is the hallmark of disease and is characterized by rapid, irregular, and arrhythmic complex involuntary movements (8–11). Moreover,The HD patients usually die within 20 years after the diagnosis either due to complications from the disease itself, suicide, heart problems or physical injury (12).

The progressive nature of the disease and the debilitating clinical manifestations impose a huge burden on the patients, their families, and healthcare systems (13–15).

The health care costs increased significantly in the late stages of the diseases. In the USA, the cost ranges from $4947 to $22,582 for private insurance and $3257 to $37,495 for Medicaid in a late stage of the disease (16).

Unfortunately,there is no cure for the disease now, However, there are pharmacological options that can alleviate the symptoms and signs of disease and prevent the disease progression and neuronal death (12,16–18).

One of the medications investigated for the treatment of HD is ethyl-eicosapentaenoic acid (ethyl-EPA) derived from omega 3 fatty acid, eicosapentaenoic acid (EPA) (19–21). Many studies had investigated its potential efficacy in numerous illness including neurological and mental health diseases (22–25).

Moreover, Ethyl-EPA had a potential efficacy in HD patients through its effect on altered lipid metabolism in Huntington disease (26,27). The experiments on mice model of HD showed enhancement of the motor activity but not neuronal death (28). However, human studies have suggested conflicting evidence with mixed results (19,20,29).

Some physicians still consider ethyl-EPA for patients with Huntington disease due to its neuroprotective effects. That’s why, this meta-analysis was conducted to critically assesses the efficacy of ethyl-EPA on HD patients and its role as an adjuvant drug for HD patients.

Methods

This study was conducted based on the recommendations of the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement (30). The

protocol was formulated prior to the study and was registered at PROSPERO International prospective register of systematic reviews (ID: CRD42016049160)

Search strategy

We searched for randomized clinical trials assessing the efficacy of ethyl-EPA for HD in 12 electronic databases including Clinicaltrials.gov, metaRegister of *Controlled Trials* (*mRCT*), WHO International Clinical Trials Registry Platform (ICTRP) to identify any ongoing studies, Google Scholar, WHO Global Health Library, POPLINE, Virtual Health Library, PubMed, Scopus, Web of Science (ISI), New York Academy of Medicine Grey Literature Report, and SIGLE (System for Information on Grey Literature in Europe).

We used the following search terms in all databases except Google Scholar: (eicosapentaenoate OR (ethyl-EPA) OR eicosapentaenoic OR timnodonic OR icosapent OR eicosapentaenoic OR padel OR eicosapentaenoate OR vascepa) AND (Huntington OR (chronic progressive hereditary chorea)).

In Google Scholar, we used advanced search with two strategies;either using "chronic progressive hereditary chorea" or "Huntington" in all words section combined with one of the words: "eicosapentaenoate "ethyl EPA" eicosapentaenoic timnodonic icosapent eicosapentaenoic epadel eicosapentaenoic vascepa".

The authors performed a manual search to retrieve any relevant papers. We searched the citations of included papers, references of relevant papers in PubMed, and relevant citations in Google Scholar.

Eligibility criteria

The papers retrieved were screened independently by three reviewers according to predefined inclusion and exclusion criteria. Our inclusion criteria were: (i) clinical trials reporting the efficacy and safety of ethyl-EPA on HD, (ii) participants should have HD clinical features and a confirmatory genetic diagnosis or a compatible family history, and (iii) all disease variants and ages of disease onset were included. Exclusion criteria were: (i) animal studies, (ii) *in vitro* studies, (iii) observational or laboratory studies, (iv) studies unreliable data set, (v) overlapped dataset, and (vi) abstract-only text or reviews, books, posters, thesis, editorial, notes, letters, case series, case reports, and conferences. Any disagreements regarding any paper between the authors were discussed to reach final decisions.

Study selection

Three independent reviewers performed an initial assessment of the retrieved references from the fore mentioned databases according to our eligibility criteria. The full texts of eligible papers were retrieved to be accurately screened by the three independent reviewers. Any disagreements were resolved by discussion and consensus among the authors till a final decision was reached.

Outcome measurement

All patients' outcomes were considered in the analysis to assess the efficacy and safety of ethyl-EPA for HD patients. We included the following: (1) unified HD rating scale (UHDRS) or any scale used to assess the disease, (2) the MRI results before and after the treatment of the patients, and (3) the side effects and complications of ethyl-EPA.

We considered the improvement of disease symptoms or signs and/or no worsening of the disease as an indication of the efficacy of the ethyl-EPA. The absence of

progression of disease was considered a good sign due to the progressive nature of the disease.

Data extraction

Three reviewers independently extracted data from eligible included references. The extracted data included study demography (title, author, year of publication, and country of patients), year of patient recruitment, participant's characteristics (age, sex, race, CAG repeats, any medication received, diagnosis of HD including family history and genetic testing, severity and grade of the disease), the dose and route of administration of placebo and ethyl-EPA, duration of treatment and follow-up, the scale used for assessment (name, the baseline score, the score after 3, 6, 12 months if available).

Quality assessment

The risk of bias in each included study was independently assessed by two reviewers using the Cochrane Collaboration's tool for assessing the risk of bias (31). It is a two-part tool, addressing seven specific domains, including randomization, allocation concealment, blinding of subjects, blinding of outcome assessors, reporting of incomplete outcome data, selective outcome reporting, and other potential sources of bias. In each domain, each study took one of three categories; ‘low risk,’ ‘high risk,’ or ‘unclear risk’ of bias (31).

Statistical analysis

We performed fixed effect model meta-analyses for each outcome using Comprehensive Meta-analysis (CMA) software version 3 (Biostat, NJ, USA) when there was more than one study for each outcome. Continuous and binary variables

were analyzed to compute pooled mean difference (MD) and risk ratio (RR), respectively. For studies that only reported mean with no measurement for the variance, we contacted the authors to give us these data. If no response from the authors, we estimated standard deviation (SD) from linear regression analysis between log (SD of the pooled studies for each outcome) against log (mean of the pooled studies for the same outcome) (17). In each outcome analysis, treatment effects were compared between per protocol (pp) and intention to treat (ITT) analysis in studies that reported both.The per protocol analysis is the analysis that includes only the remaining patients at the end of the experiment while the intention to treat analysis analyze the originally allocated patients regardless of lost to follow-up patients. Both should be done in clinical trials to avoid bias (32).

We assessed statistical heterogeneity between studies using the Higgins' Chi-square (Chi^2) and I-squared (I^2) statistic. When P-value of a Chi^2 test was < 0.1 and/or I^2 test > 50%, it was considered a significant for the presence of heterogeneity (33,34). If there was no study reported pre/post correlation, we made a sensitivity analysis by assuming several values of correlation (35,36). The statistical significance was considered if the P-value was 0.05 (two-tailed test) or its 95% confidence interval (95% CI) did not overlap with the original one.

Ferreira et al. (19) reported their results using full analysis set (FAS) and modified full analysis set (mFAS) while Puri et al. (37) used PP and ITT. Sensitivity analysis was done using each design separately for the analysis. The analysis was done first using per protocol analysis with FAS then with mFAS then we removed Ferreira et al. from the analysis.

For the analysis at 12 months, two studies were only included and one of them had six months' open label phase which was reflected on the quality assessment result. We observed that this study had the largest weight in the meta-analysis which may affect the results of the analysis. That's why We did a *post- hoc analysis* for the meta-analysis at 12 months.. Quality effect model meta-analysis was performed to account for these issues . We assessed the quality of the studies by quality assessment tool proposed by Doi et al (38) then applied the method reported in Doi et al study (38). Post-hoc meta-analysis was conducted in Microsoft Excel 2016.

Results

Literature search

The electronic search yielded 204 references from the 12 databases. After excluding the duplicates and title/abstract screening, we had nine relevant papers for full-text screening and only five papers fulfilled the inclusion criteria. The manual search did not result in additional papers (Figure 1).

In the end, we had five RCTs for the systematic review but only four papers could be included in the meta-analysis.

Study characteristics

782 cases (ethyl-EPA = 391, placebo = 391) were included in the meta-analysis and were recruited from UK, Germany, Portugal, Spain, Italy and Austria, USA, Canada, and Australia. The HD patients' age ranged from 50 to 63 with no significant difference in age in all trials between ethyl-EPA and placebo groups Table 1.

All studies used purified ethyl-EPA in a dose range 1-2 gm /day. For placebo, all studies used a sub-laxative dose of liquid paraffin. All trials were double blinded randomized trials.

The number of CAG repeats in the included patients ranged from40 to 49 Table 1. There was no significant difference of CAG repeats between the placebo and ethyl-EPAin all studies.

Quality assessment

The results of the quality assessment are shown in Figure 2. Four RCTs had low risk of bias; TRENDHD had attrition and other bias illustrated in supplementary Table 1.

Efficacy and safety of ethyl-EPA

Total motor score (TMS)

The fixed effect model meta-analysis of studies at six months showed no significant improvement of the TMS on patients receiving ethyl-EPA compared to placebo [MD = -0.527, 95% CI (-1.67; 0.61), *P = 0.365*] with no significant heterogeneity [*P = 0.454, I^2 = 0%*]. Comparison of treatment effects between per protocol analysis and intention to treat analysis yielded the same insignificant effect of ethyl-EPA on TMS compared to placebo (Figure 3A, 3B).

Sensitivityanalysis was done by removing of Ferreira et al. (19) that used least mean squares for reporting their results but it did not produce any significant changes in the analysis (Supplementary Figure 1A, B).

In contrast to the 6 months' analysis, the fixed effect model meta-analysis at 12 months yielded significant results [PP (MD = -2.72, 95% CI (-4.76; -0.68), *P = 0.009*) and ITT (MD = -2.23, 95% CI (-4.09; -0.38), *P = 0.018*)] with no significant heterogeneity [*P = 0.764, I^2 = 0%*] (Figure 4A, B).

Post - hoc analysis

Despite the significant results of fixed model meta-analysis of TMS after 12 months, we did a post-hoc analysis because the TRENDHD study constituted 86% of the weight in the meta-analysis. TRENDHD (20) study has both attrition and detection bias as it included open label six months phase. We did quality effect model meta-analysis which takes in consideration the quality of included studies in the analysis. In case of using per protocol group, MD was -2.36 with 95% CI (-0.56; -4.48) while for ITT group, MD was -1.96 with 95% CI (-0.004; -3.92).

Total motor score- 4 (TMS-4)

TMS-4 is a shortened version of the total motor score (TMS) that was used for assessment of motor improvement in three studies (39). Pooling of these studies at six months did not show any significant improvement of the score in the treatment group compared to placebo group [MD = -0.82, 95% CI (-1.83; 0.19), *P = 0.11*] Figure 5A. Sensitivity analysis yielded the same insignificant results Figure 5 and supplementary figure 2.

At 12 months, the TMS-4 was significantly improved in treatment group compared to placebo group [MD = -2.225, 95% CI (-3.842; -0.607), *P = 0.007*] with no significant heterogeneity detected [$P = 0.293$, $I^2 = 9\%$] in case of per protocol analysis Figure 6A. When only including intention to treat analysis with other study, the mean difference was -1.831 [95% CI(-3.427; -0.235), *P = 0.025*] with no detected heterogeneity [$P = 0.502$, $I^2 = 0\%$] Figure 6B.

Post hoc analysis

Quality effect model meta-analysis yielded the same significant results. For PP analysis mean difference was -2.58 with 95% C.I (-0.62; -4.54);for ITT analysis, mean difference was -1.64 with 95% C.I (-0.32; -3.60).

Maximal chorea score

The score did not improve significantly after six months in patients receiving ethyl-EPA compared to placebo [MD = 0.345, 95% CI (-0.907; 0.218), *P = 0.23*]; no heterogeneity [*P = 0.55, I^2 = 0%]* Supplementary Figure 3A while it significantly improved in ethyl-EPA group after 12 months [MD = -1.013, 95% CI (-1.793; -0.233), *P = 0.011*] with no heterogeneity (*P = 0.423, I^2 = 0%*) Supplementary Figure 3B.

Post hoc analysis

Unlike the fixed effect model, quality effect model yielded insignificant results [mean difference = -0.99, 95% CI (0.97; -2.95)].

Stroop color naming

At six months, no significant improvement was observed in patients receiving ethyl-EPA compared to placebo group [MD = -0.496, 95% CI (-1.415; 0.423), *P = 0.290*] with no detected heterogeneity [*P = 0.698, I^2 = 0%*] Supplementary Figure 4A. Unlike other outcomes, the Stroop color naming test score did not improve after 12 months [MD = -0.781, 95% CI (-2.382; 0.820), *P = 0.339*] with no significant heterogeneity [*P = 0.698, I^2 = 0%*] (Supplementary Figure 4B).

Symbol digital modality

Patients receiving ethyl-EPA did not improve significantly after six months compared to placebo group [MD = -0.496, 95% CI (-1.415; 0.423), *P = 0.290*].

Clinical global impression scale

There was no significant improvement nor change in the symptoms or signs of the included patients in the ethyl-EPA group compared to the placebo group [RR = 1.056, 95% CI (0.78; 1.44), *p = 0.73*], and [RR = 0.9, 95% CI (0.76; 1.07), *p = 0.24*], respectively Supplementary Figure 5B. Moreover, there was no significant risk for

worsening of symptoms and signs in patients receiving ethyl-EPA compared to those receiving placebo [RR = 1.183, 95% CI (0.861; 1.627), *p = 0.3*] Supplementary Figure 5B.

h.

Adverse events

There are reported side effects in three studies (21-22,41). Only one study reported the side effects at 6 and 12 months (20) while others reported side effects at 6 months . Diarrhea, fall, nasopharyngitis, and depression were reported in the three studies (21-22,41). There was no significant difference between ethyl-EPA and placebo regarding the risk for diarrhea, fall, nasopharyngitis and depression Supplementary Figure 6 at 6 months with risk ratio of 0.92 (0.561; 1.493), 0.385 (0.140; 1.062), 1.486 (0.604; 3.661) and 1.218 (0.62; 2.41), respectively with no significant heterogeneity ($P = 0.70$, $I^2 = 12\%$).

No study reported specific side effects related to ethyl-EPA. Other reported side effects are summarized in supplementary table 2.

Qualitative synthesis

Puri et al. was excluded from the analysis because they used only MRI to assess the efficacy of ethyl-EPA unlike other studies in the analysis that used UHDRS subscales (40).

The Puri et al. study demonstrated how the ethyl-EPA affected the cerebral atrophy in HD patients (40). The study performed double-blind sagittal three-dimensional T1 MRI for imaging of local and global brain atrophy in both ethyl-EPA and placebo groups at baseline, six months, one year of follow-up. They found a significant decrease of progressive brain atrophy at six months in ethyl-EPA treated patients (mean change = -0.32, standard error [SE] = 0.15) versus placebo-treated patients

(mean change = - 0.615, SE = 0.081, P < 0.05), however,in the second six months, the change in both arms was the same. Surprisingly, the overall reduction of global brain atrophy after one year of treatment in ethyl-EPA treated patients was insignificant (mean change = -0.75, SE =0.23) versus placebo-treated patients (mean change = -1.22, SE = 0.2, P < 0.06). The local analysis revealed a reduction of the regional atrophy at the head of caudate nucleus and posterior thalamus after one year compared to the baseline in ethyl-EPA treated patients. This was consistent with another study that revealed an increase in the ventricular size in placebo-group as a sign of progressive atrophy compared to the ethyl-EPA group that showed decreased ventricular size (29).

Discussion

This study was set out with the aim of assessing the efficacy of ethyl-EPA as an adjuvant treatment for Huntington disease.Furthermore, we also investigatedhow it affects the progressive brain atrophy in HD.

The most obvious finding to emerge from the analysis is that the administration of ethyl-EPA for 12 months with a dose of one to two grams resulted in a significant improvement of scores related to the motor functions of the patient including the total motor score [MD = -2.23, 95% CI (-4.09; -0.38), P = 0.018], total motor score-4 [MD = -2.225, 95% CI (-3.842; -0.607), P = 0.007], and the maximal chorea score [MD = -1.013, 95% CI (-1.793; -0.233), P = 0.011]. Contrary to expectations, this study did not find a significant improvement on the scales related to cognitive function including Stroop color naming test and the symbol digital modality test.

Previous literature proved the significance of EPA on the cognitive function in elderly (22,24,41) but nothing was found to explain why there was no effect on cognition of

Huntington patients after 12 months. In addition, Puri et al. reported significant worsening of behavioral changes in ethyl-EPA group versus placebo group in ITT group (37). Moreover, after six months, ethyl-EPA failed to produce any significant improvement of any scales in the patients.

The improvement of motor function after 12 months is consistent with experimental evidence in mice that indicated administration of ethyl-EPA in YAC128 mouse model improved motor functions (28). Van Raamsdonk et al. delivered oral ethyl-EPA for six months and found a significant modest improvement of the motor function (28). Also, Clifford et al. used essential fatty acid for successfully delaying the progression of motor symptoms in the experimental mice (42). This is contrary to human studies which only had a significant effect after 12 months (20,37).

Despite this improvement of the motor score, the improvement failed to have a significant effect on the clinical global or total functional capacity after 12 months. In all RCTs included in our analysis, the authors used semi-subjective UHDRS subscales for assessment of the efficacy of ethyl-EPA (43). Motor subscales of UHDRS failed to show any significant improvement after six months. The subjective nature of the scale may explain this variability. Vaccarino et al. suggested that scores like saccade velocity and tongue protrusion had a high probability to be scored 4 or 0 than middle options while chorea, gait, and rigidity were less scored high as 3,4 (44). Moreover, these scores are less sensitive to changes to motor severity especially in more severe cases (44). In addition, another study recommended the test to be done annually for follow-up to be sensitive to motor changes (43). However, this evidence is contradicted by other studies that recommended using the UHDRS for research purposes (43,45,46). In addition, other trials used the UHDRS after 12 weeks and it could detect the improvement within this short duration (47,48).

For trials assessing ethyl-EPA, MRI results at six months were more reliable than UHDRS motor scores (29). One of our included studies has assessed the outcome at six months by both MRI and UHDRS (29). MRI was more sensitive and reliable to brain changes at six months.

Puri et al. investigated the effect of number of CAG repeats on the significant motor outcome and found that ethyl-EPA has more significant effect on patients with lower CAG repeats than those with high CAG repeats (37). They suggested that ethyl-EPA may be beneficial for patients with low CAG repeat and delayed onset which needs further investigation.

The studies included in the analysis were assessed for bias that may affect the interpretation of results. Twelve months' results of the TREND-HD study (20) were including six months open-label phase. This could lead to attrition and detection bias. Detection bias were excluded by investigators because the improvement occurred only in the ethyl-EPA group, not in the placebo but still the results remained inconclusive. Puri et al. study (37) did not report how they did sequence generation. No detectable bias were found in other studies. That's why, our results should be interpreted cautiously especially at 12 months.

Our hypothesis implied that this improvement is not only symptomatic but also related to the delayed direct effect of ethyl-EPA on the brain atrophy as evidenced by the double-blinded MRI studies that become apparent after 12 months (29,40). These two double-blinded studies suggested there is a significantly less regional atrophy at the head of caudate nucleus and posterior thalamus compared to placebo in patients receiving ethyl-EPA compared to patients receiving placebo (29,40).

Ethyl-EPA interferes with different reported mechanisms of neuronal degeneration of HD Supplementary Figure 7. A possible mechanism is activated immune response

433 releasing cytokines mainly interleukins that activate apoptotic pathways that will
434 eventually result in neuronal death especially striatal cells(49). These mechanisms
435 were interfered by the strong anti-inflammatory effect of ethyl-EPA. In addition, EPA
436 can protect neuronal cells by inhibiting interleukin - 1 induced hippocampal cells
437 apoptosis (26,50).
438 Another mechanism implicated in neuronal death in HD is activation of the c-Jun N-
439 terminal pathway (JNK pathway) which is considered as one of the main pathways
440 involved in the neuronal death (2,50,51). This pathway is either activated by
441 glutamate-mediated excitotoxicity on N-methyl-D-aspartate receptors (NMDA), by
442 inflammatory cytokines or by nuclear polyglutamine aggregates (49,52). EPA was
443 found to interfere with the above-proposed mechanism of neuronal degeneration of
444 HD. It acts against many cytokines and lipopolysaccharides induced activation of
445 JNK pathway (53). It can also decrease the activity of AP-1 and p53 in epidermal and
446 mesangial cells but its effect on the pathology of the brain is still inconclusive (54).
447 Experimental studies proved that EPA acts as a precursor of brain phospholipids
448 (27,55) which is depleted by abnormal Huntingtin protein (27). A study proved its
449 effectiveness in relieving oxidative stress in mitochondria (56).
450 In addition to its effect on brain atrophy, there was no significant side effects in the
451 ethyl-EPA group making it a perfect candidate for long term therapy.
452
453 **Recommendations for further trials**
454 We recommend more trials to test the effect of EPA as a preventive treatment in
455 prodromal HD to delay the onset of the disease. The effect of ethyl-EPA on the brain
456 atrophy should not be ignored and more studies should be done. More trials with

larger sample size and longer duration of treatment are needed to assess the real efficacy of ethyl-EPA after 12 months.

Limitation of the review

We faced some limitations during the study. Firstly, the few number of RCTs performed and small samples of the included studies led to a decreased power of the analysis and inability to achieve conclusive results. Another limitation was a small number of studies continued the trial for 12 months. More studies with larger sample size are needed to prove its effectiveness and to assess if these brain improvements will take time until it becomes evident on the clinical profile of the patients and if this is the cause of significant improvement only at 12 months not at 6 months.

Conclusion

Our results indicated a significant improvement of motor scores only after 12 months with no effect on other scales. However, these results should be interpreted cautiously.

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Author Contribution Statement:

Nguyen Tien Huy, Kenji Hirayama: Principal investigators, revised the analysis and data and reviewed the manuscript.

Sara Morsy, Nguyen Tien Huy, Kenji Hirayama: Formulation of the research idea

Doaa Saeed Mahmoud El-Basiony, Hossam Idrees Ahmed Hassan, Ahmed Abdelaziz Eisa, Cao Thị Anh Ngoc, Nguyen Phu Dang, Sara Morsy, Mohamed F Doheim, Samar M Khalil: Screening, Extraction, Quality assessment, Characteristics table.

Sara Morsy, Mohamed Gomaa, Samar M Khalil: Statistical analysis

Sara Morsy: Post-hoc analysis, Writing and Figures

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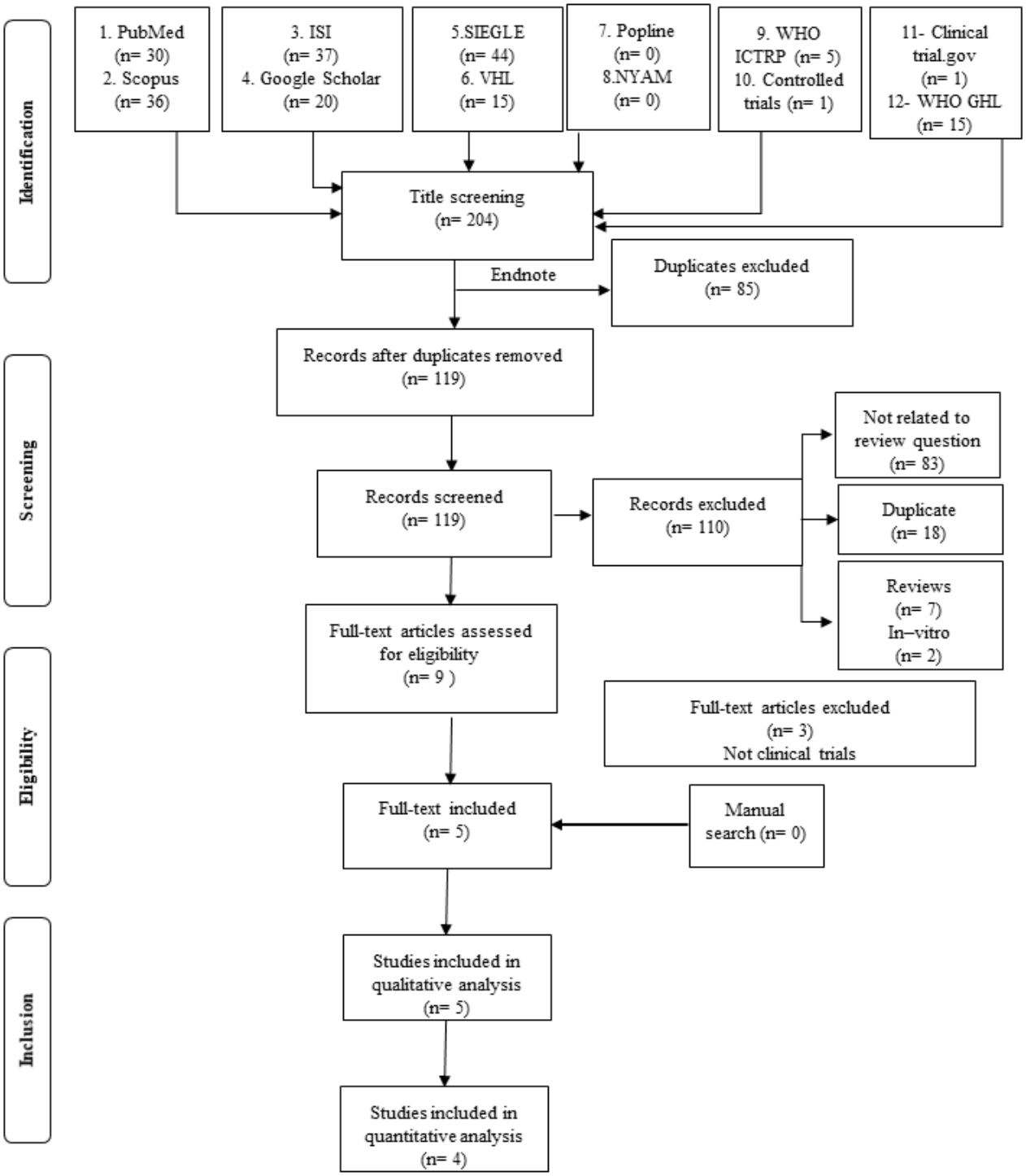


Figure 1. PRISMA checklist illustrating the flow of the review

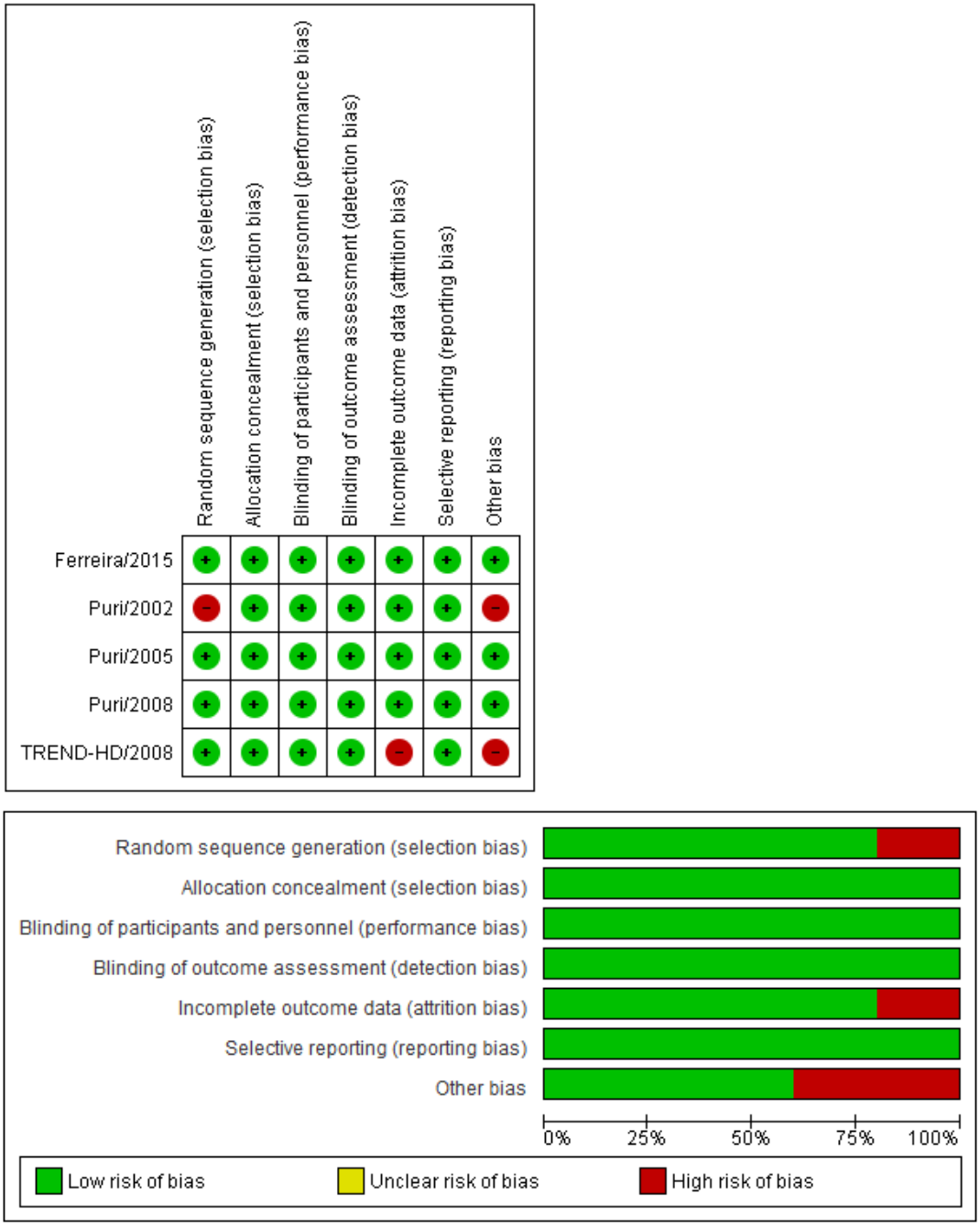


Figure 2. Quality assessment results as assessed by Cochrane risk of bias assessment tool. Red = high risk, blank = unclear, green = low

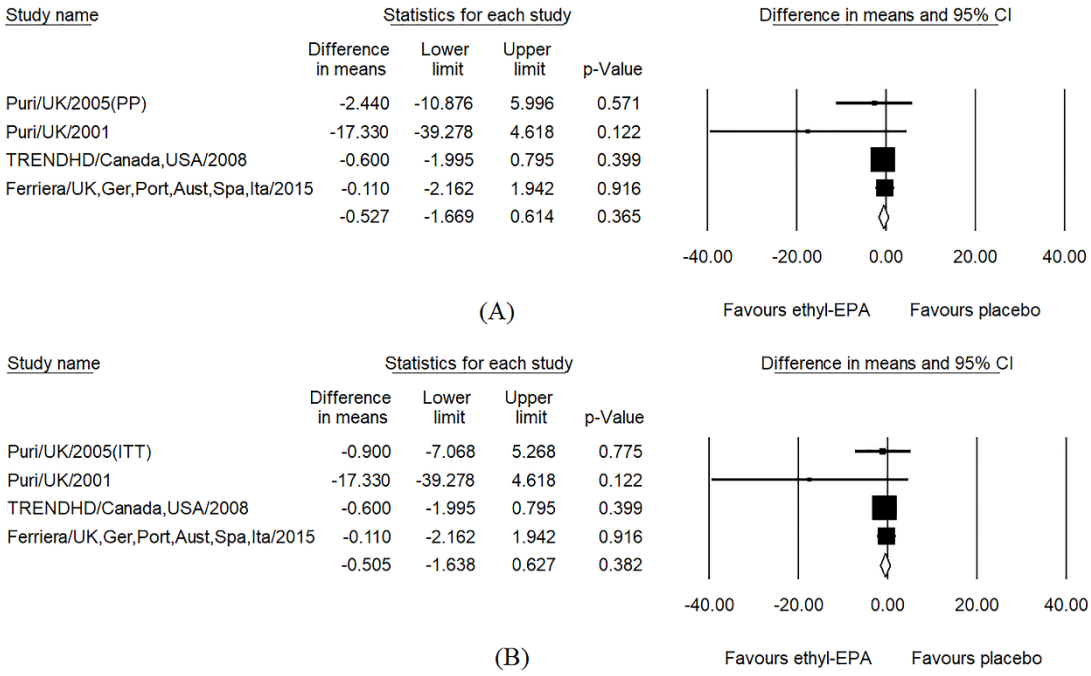


Figure 3. Fixed effect meta-analysis of the mean difference of scores of total motor score (TMS) between placebo and ethyl-EPA at 6 months. Each study is represented by points which have a size corresponding to its weight in the analysis. Mean and 95% confidence interval (C.I) are used for the overall effect size represented by diamond. We did a separate analysis for per protocol (A) and intention to treat analysis (ITT, B) used in Puri et al. 2005.

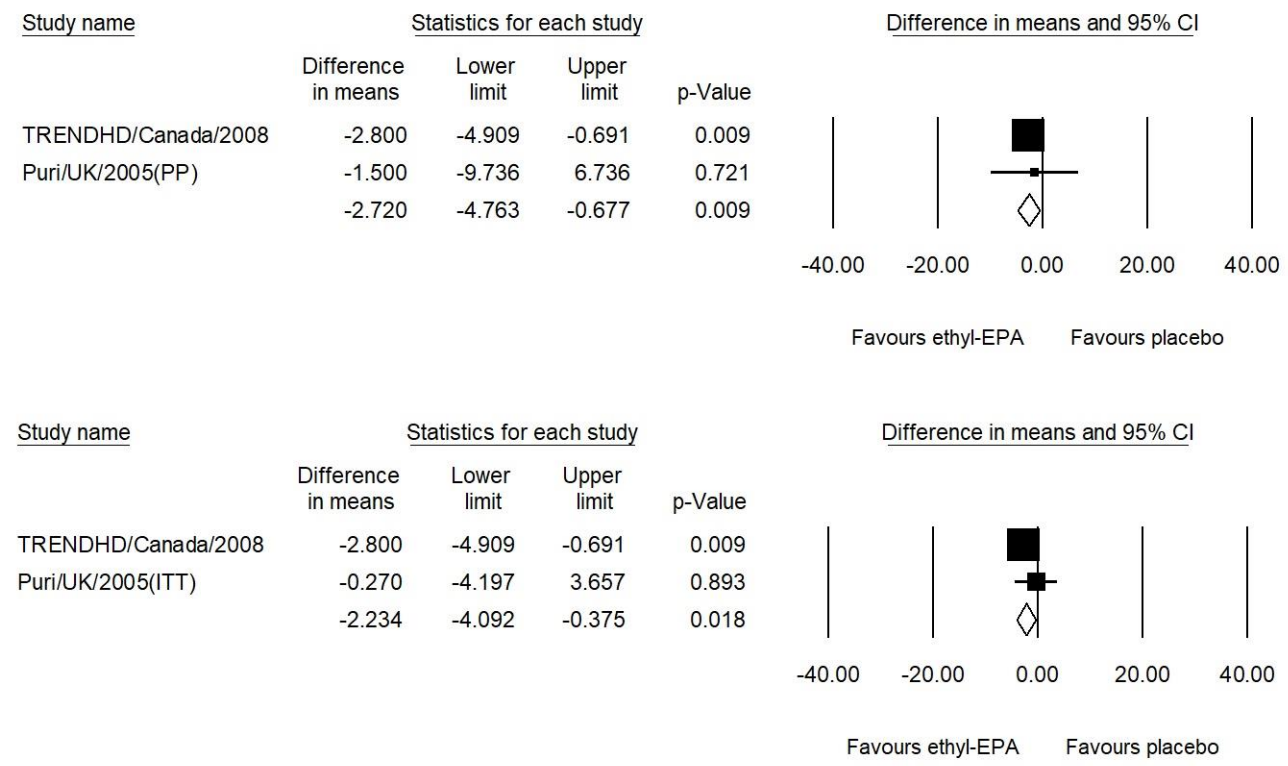


Figure 4. Fixed effect meta-analysis of the mean difference of scores of total motor score (TMS) between placebo and ethyl-EPA at 12 months. Each study is represented by points which have a size corresponding to its weight in the analysis. Mean and 95% confidence interval (C.I) are used for the overall effect size represented by diamond. We did a separate analysis for per protocol (A) and intention to treat analysis (ITT, B) used in Puri et al. 2005.

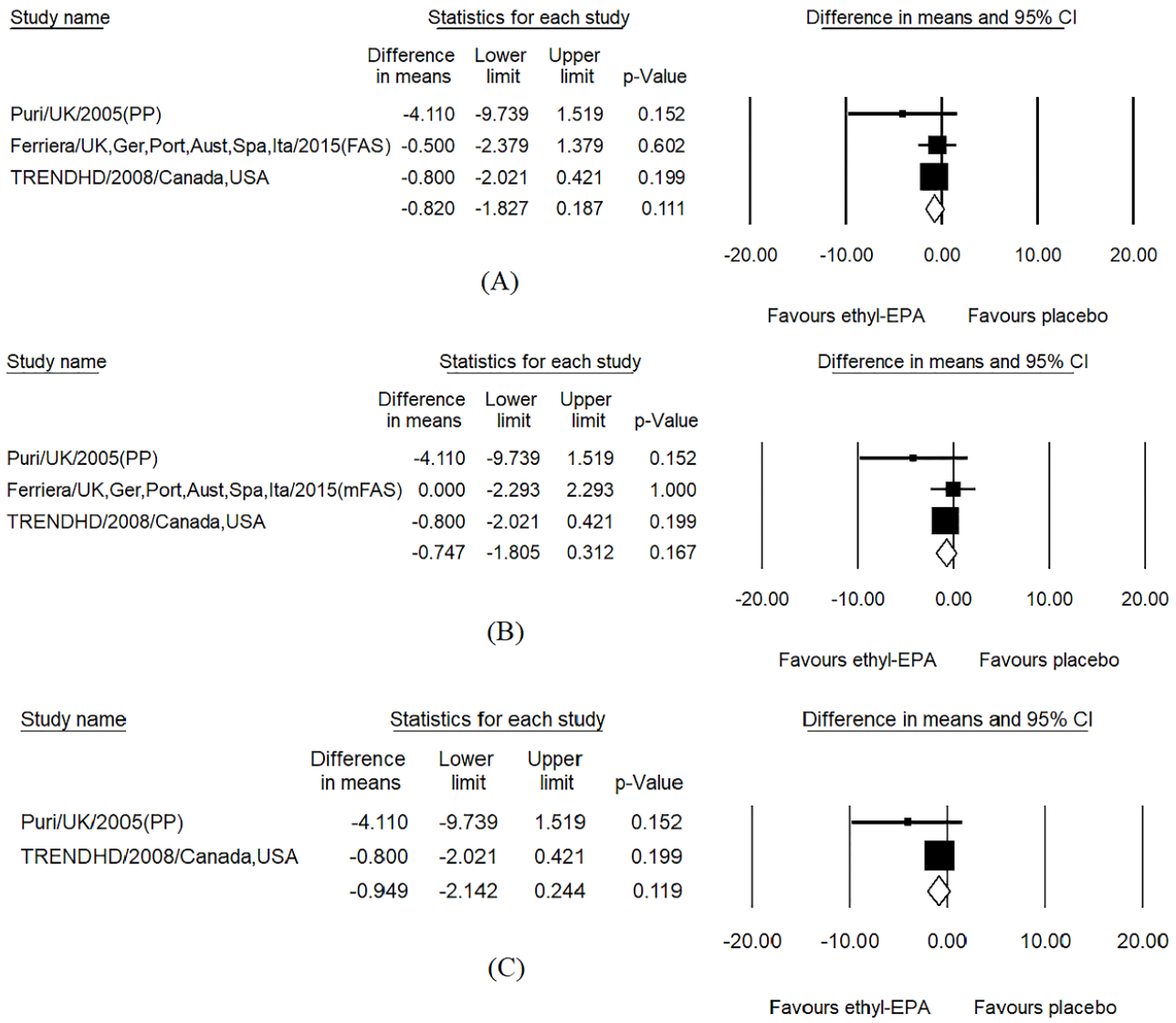


Figure 5. Meta-analysis of the mean difference of scores of shortened version of total motor score (TMS-4) between placebo and ethyl-EPA at 6 months. Each study is represented by points which have a size corresponding to its weight in the analysis. Mean and 95% confidence interval (C.I) are used for the overall effect size represented by diamond. We did a separate analysis for (A) only per protocol analysis (PP) of Puri et al 2005, and full set analysis (FAS) of Ferreira et al 2015**, (**B) only per protocol analysis (PP) of Puri et al., 2005 and modified full set analysis (mFAS) of Ferreira et al 2015, and (C) only per protocol analysis (PP) of Puri et al 2005.

~~**Table 1. Characteristics of the included studies.**~~

~~ethyl-EPA: ethyl-eicosapentaenoic acid; CAG: cytosine-adenine-guanine;UHDRs: unified Huntington disease rating scale; SD: standard deviation.~~

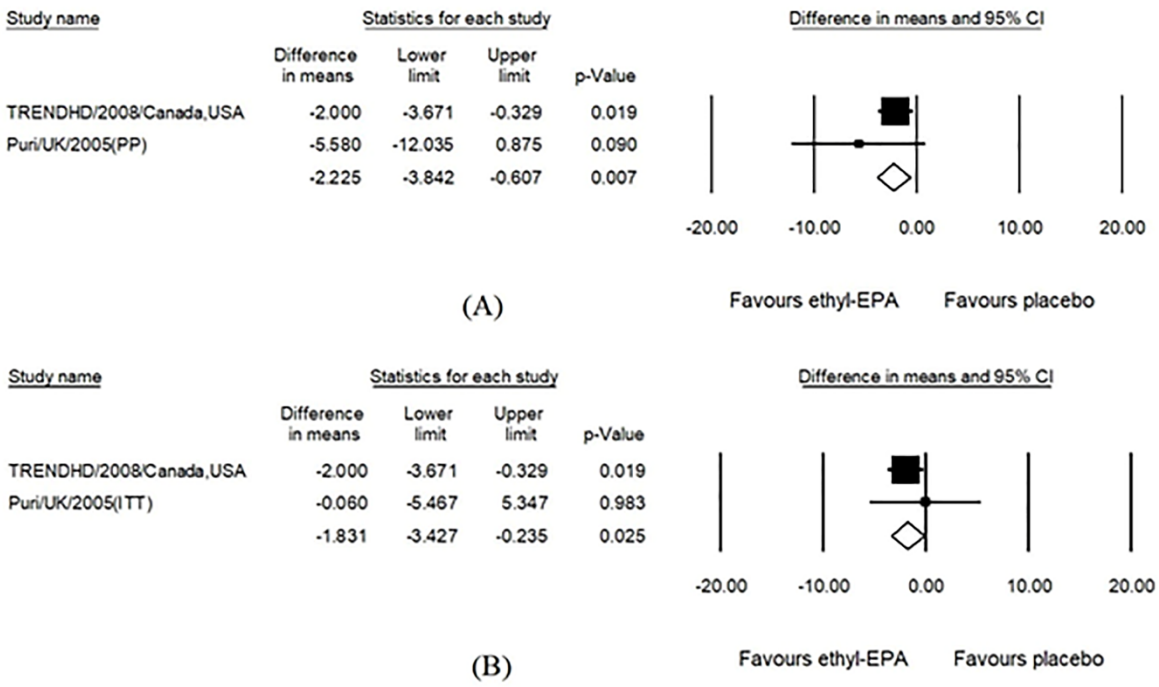
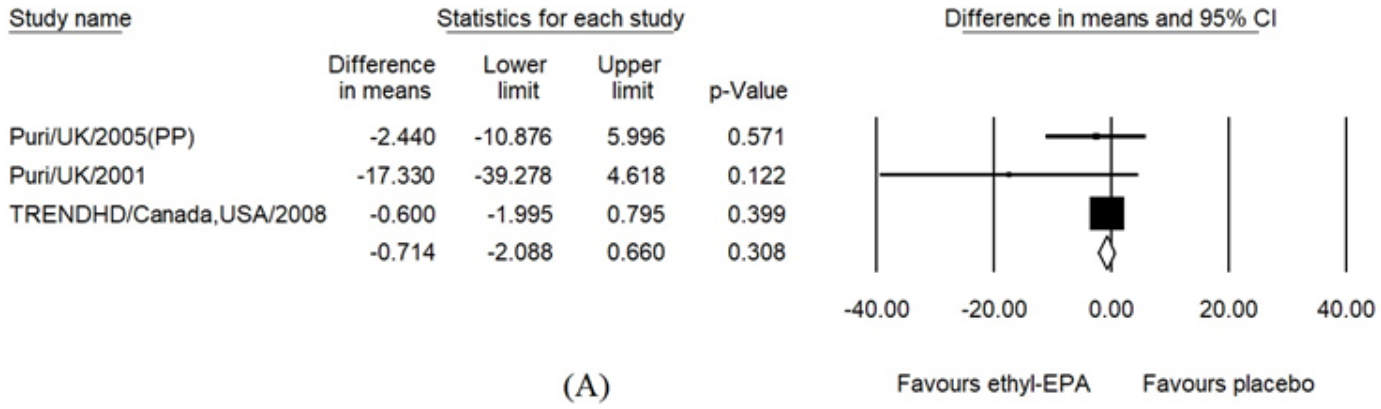


Figure 6. Fixed effect meta-analysis of the mean difference of scores of shortened version of total motor score (TMS-4) between placebo and ethyl-EPA at 12 months. Each study is represented by points which have a size corresponding to its weight in the analysis. Mean and 95% confidence interval (C.I) are used for the overall effect size represented by diamond. We did a separate analysis for per protocol (A) and intention to treat analysis (ITT, B) used in Puri et al. 2005.

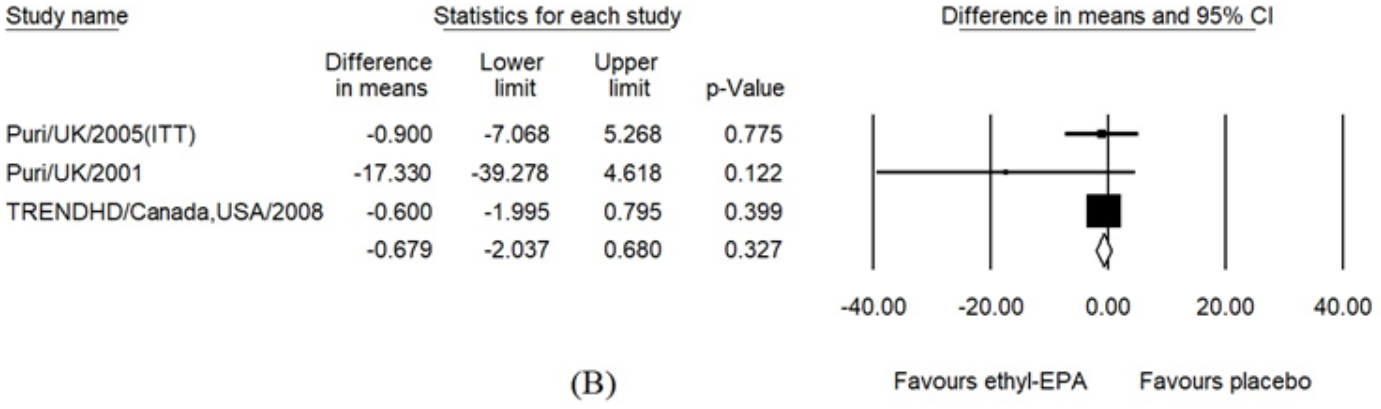
Supplementary figures

Supplementary figure 1.:

Meta-analysis of the mean difference of scores of shortened version of total motor score (TMS-4) between placebo and ethyl-EPA at 6 months after exclusion of Ferreira et al./2015. Each study is represented by points which have a size corresponding to its weight in the analysis. Mean and 95% confidence interval (C.I) are used for the overall effect size represented by diamond. A, Forest plot using per protocol (PP), B, Forest plot using intention to treat analysis (ITT)

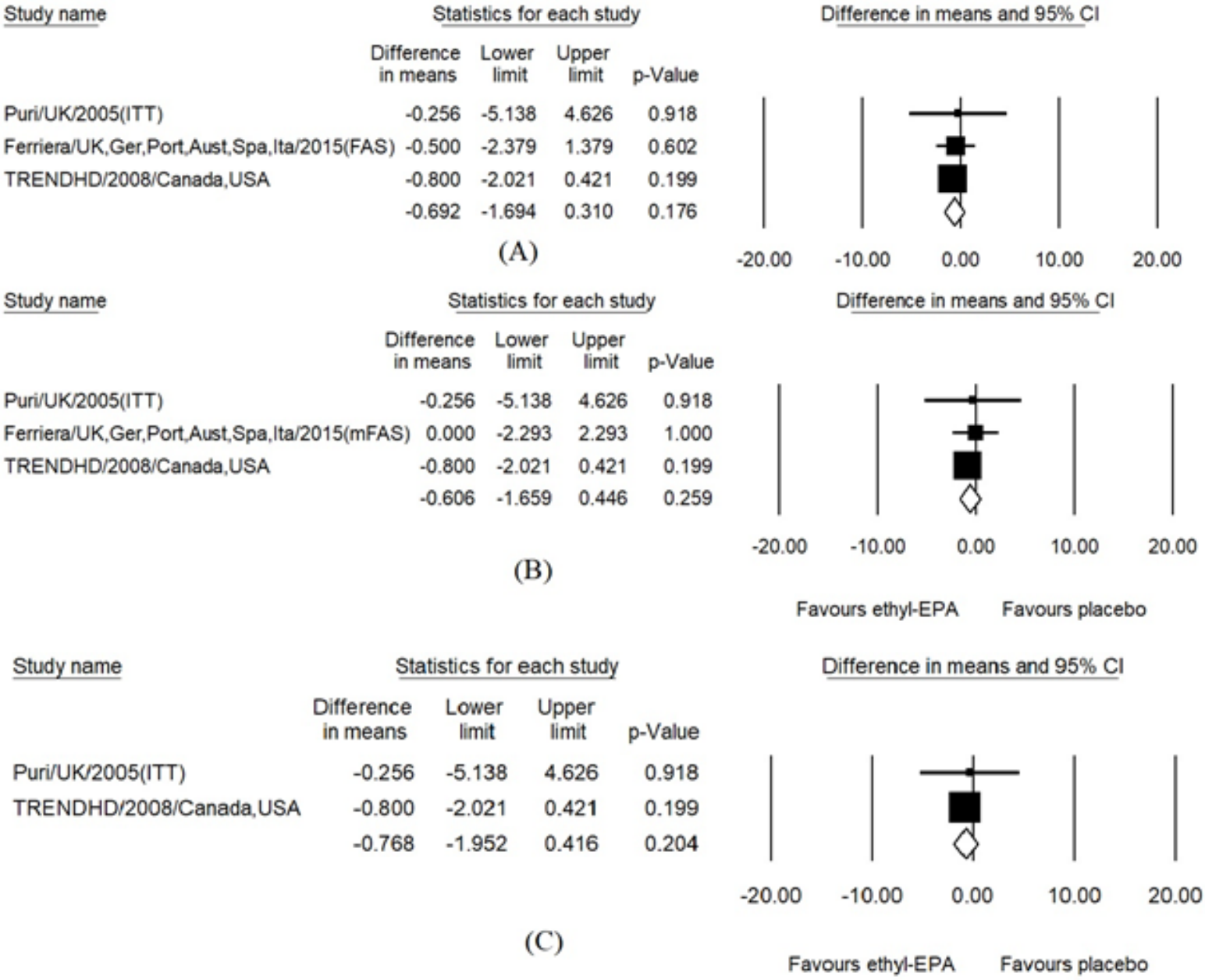


(A)

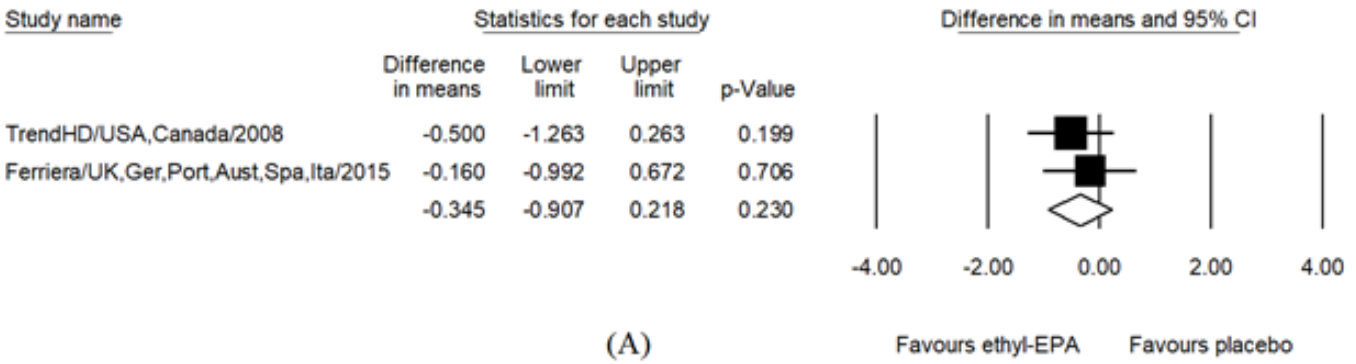


(B)

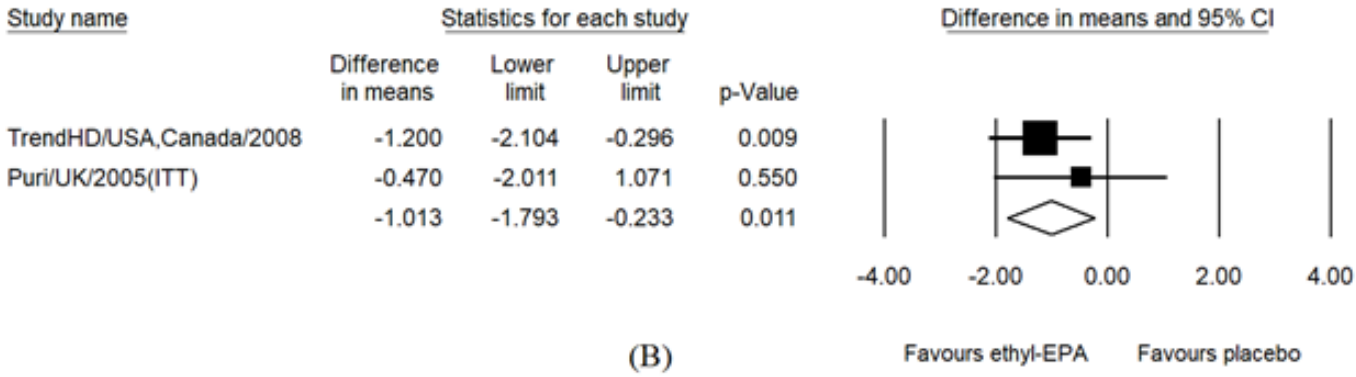
Supplementary figure 2: Meta-analysis of the mean difference of scores of shortened version of total motor score (TMS-4) between placebo and ethyl-EPA at 6 months. Each study is represented by points which have a size corresponding to its weight in the analysis. Mean and 95% confidence interval (C.I) are used for the overall effect size represented by diamond. We did a separate analysis for (A) only intention to treat analysis (ITT) of Puri et al 2005, and full set analysis (FAS) of Ferreira et al 2015**, (**B) only intention to treat analysis (ITT) of Puri et al., 2005 and modified full set analysis (mFAS) of Ferreira et al 2015, and (C) only intention to treat analysis (ITT) of Puri et al 2005.



Supplementary Figure 3. Forest plots of the mean difference of scores of maximal chorea score and stroop color naming test between placebo and ethyl-EPA at 6 (A) and 12 (B) months

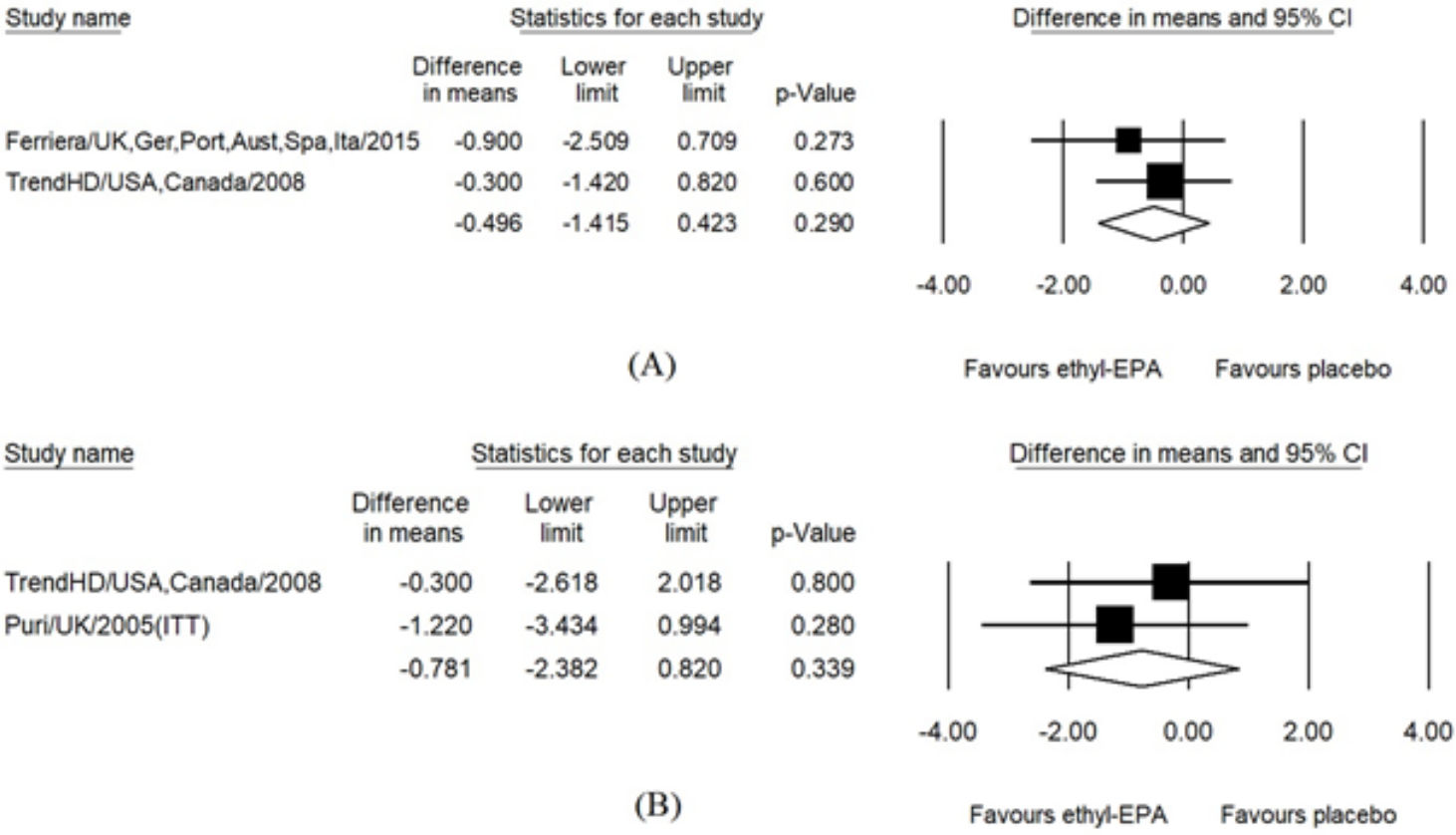


(A)

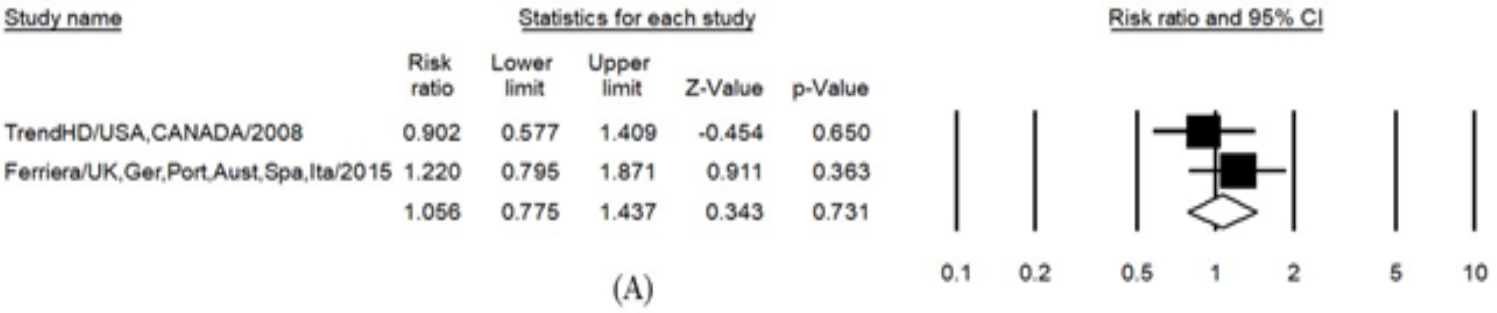


(B)

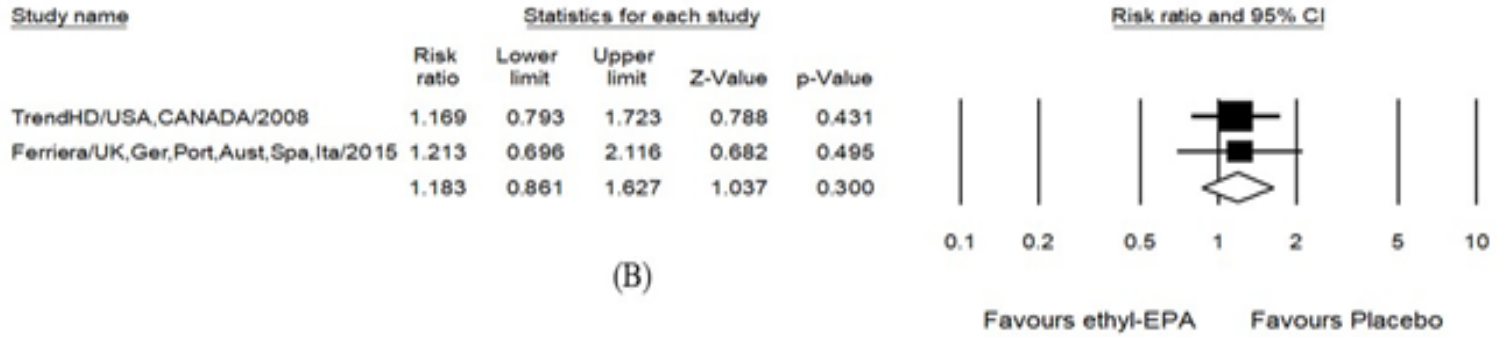
Supplementary Figure 4. Meta-analysis of the mean difference of scores of stroop color naming test between placebo and ethyl-EPA at 6 (A) and 12 (B) months



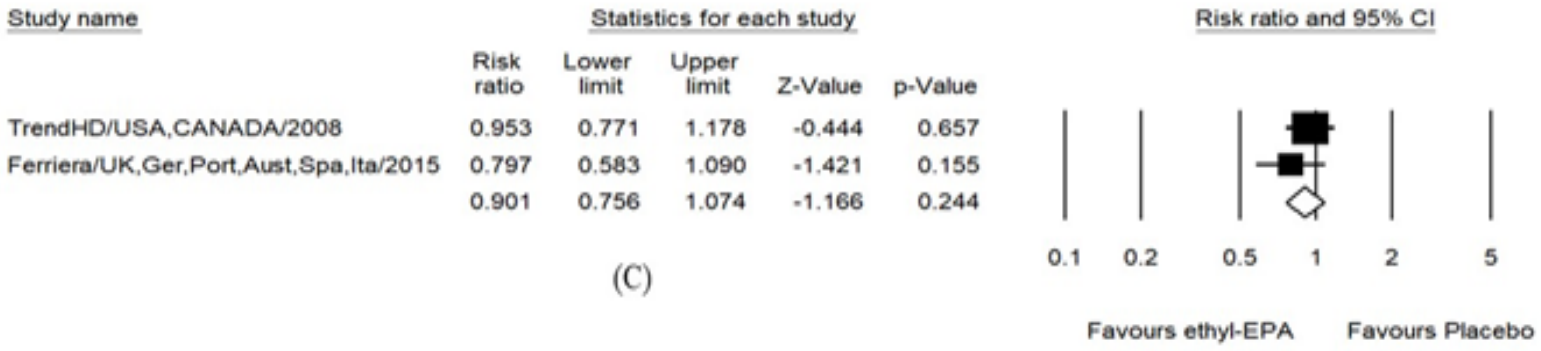
Supplementary Figure 5. Meta-analysis forest plots of the risk ratio of each category of clinical global impression scale at six months, (A) improved, (B) worsened, (C) no change,



(A)

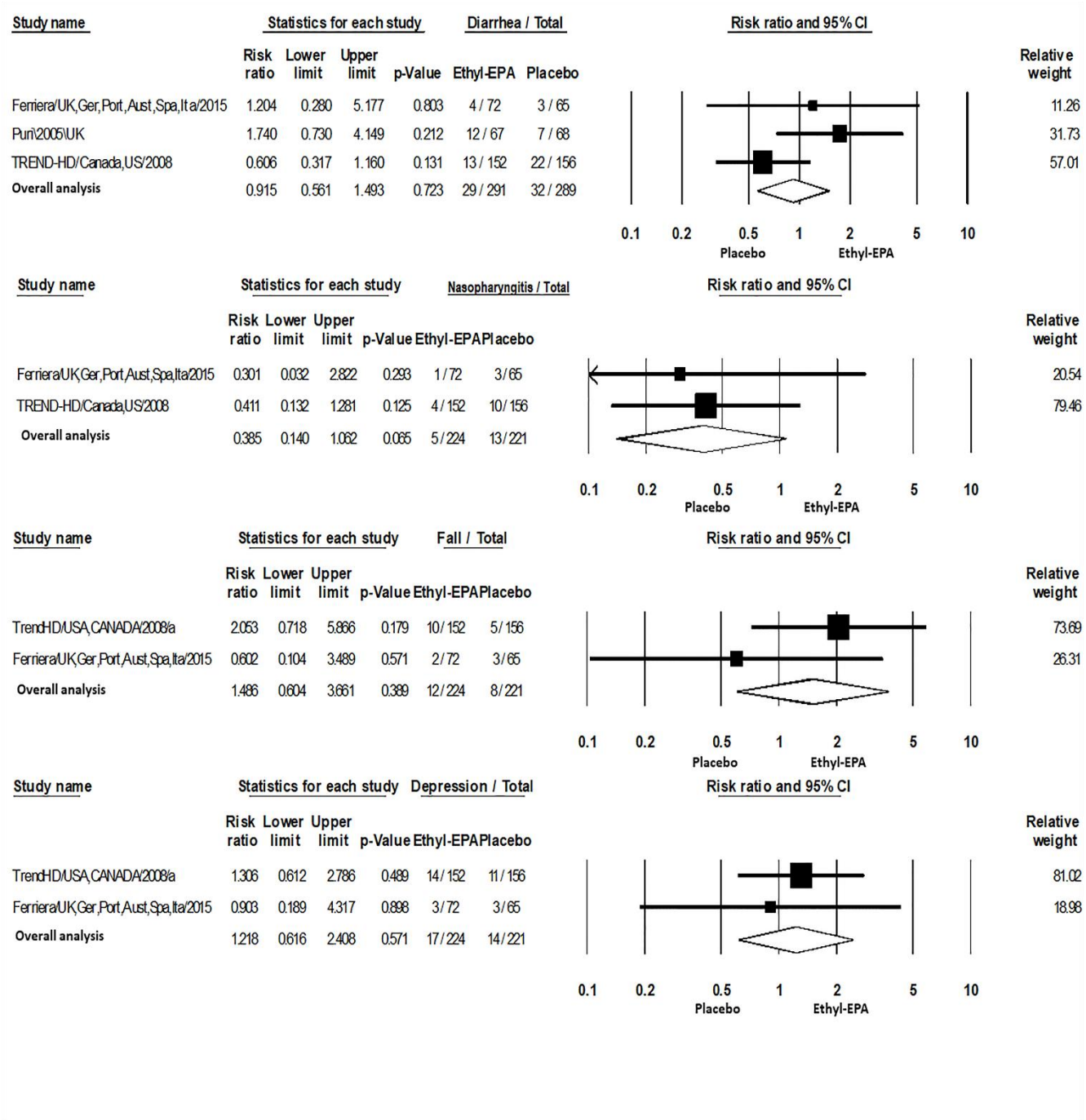


(B)



(C)

Supplementary figure 6. Forest plot of risk ratio (RR) of diarrhea, nasopharyngitis, fall, depression in ethyl - EPA group compared to placebo group at six months



Supplementary Figure 7 Summary of the possible mechanisms of neurons' death in HD.

NMDA receptor: The N-methyl-D-aspartate receptor, JNK: c-Jun N-terminal kinas