ELEVATED BLOOD HARMANE (1-METHYL-9H-PYRIDO[3,4-*B*]INDOLE) CONCENTRATIONS IN ESSENTIAL TREMOR

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Abstract

Essential tremor (ET) is a widespread late-life neurological disease. Genetic and environmental factors likely play an etiological role. Harmane (1-methyl-9H-pyrido[3,4-*b*]indole) is a potent tremor-producing neurotoxin. In 2002, we demonstrated elevated blood harmane concentrations in an initial sample of 100 ET cases compared to 100 controls. Between 2002 and 2007, we assembled a new and larger sample of ET cases and controls. We now attempt to replicate our previous findings. Cases and controls were frequency-matched on age, gender, and race. Blood harmane concentrations were quantified by high performance liquid chromatography. Subjects comprised 150 ET cases and 135 controls (mean age 65.3 ± 15.5 vs. 65.5 ± 14.2 years, $p = 0.94$). Mean log blood harmane concentration was ~50% higher in cases than controls (0.50 ± 0.54 g^{-10}/ml vs. 0.35 ± 0.62 g^{-10}/ml, $p = 0.038$). In a logistic regression analysis, log blood harmane concentration was associated with ET ($OR_{adjusted}$ 1.56, 95% CI 1.01 - 2.42, $p = 0.04$), and odds of ET was 1.90 (95% CI 1.07 - 3.39, $p = 0.029$) in the highest vs. lowest log blood harmane tertile. Log blood harmane was highest in ET cases with familial ET (0.53 ± 0.57 g^{-10}/ml), intermediate in cases with sporadic ET (0.43 ± 0.45 g^{-10}/ml) and lowest in controls (0.35 ± 0.62 g^{-10}/ml) (test for trend, $p = 0.026$). Blood harmane appears to be elevated in ET. The higher concentrations in familial ET suggests that the mechanism may involve genetic factors.

Keywords

essential tremor; epidemiology; beta-carboline alkaloid; harmane; toxin; environmental risk factors

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Introduction

Essential tremor (ET), characterized by action tremor of the hands, is a widespread late-life neurological disease that is present in 4.0% of individuals aged ≥ 40 years and 8.7% in individuals in their ninth decade (Benito-Leon et al., 2003; Dogu et al., 2003). As such, it is one of the most common neurological disorders (Louis et al., 1998b). In addition to action tremor, patients may exhibit other signs, including cognitive impairment (Benito-Leon et al., 2006), and gait ataxia and incoordination (Singer et al., 1994). Both genetic (Gulcher et al., 1997; Higgins et al., 1997) and non-genetic (environmental factors)(Jiménez-Jiménez FJ and B., 2007; Louis, 2001; Salemi et al., 1998) are likely to play a role in disease etiology.

The β-carboline alkaloids are a group of neurotoxins that produce tremor. Laboratory animals injected with high doses acutely exhibit action tremor that resembles ET (Fuentes and Longo, 1971; Zetler et al., 1972). Human volunteers exposed to high doses display a coarse, reversible action tremor (Lewin, 1928).

Harmane (1-methyl-9H-pyrido[3,4-*b*]indole) is among the most potent tremor-producing β-carboline alkaloids; 38 mg/kg of subcutaneously administered harmane produces tremor in mice (McKenna, 1996). Harmane is very lipid soluble (Zetler et al., 1972), and broadly distributed within the rat brain (Anderson et al., 2006; Matsubara et al., 1993; Moncrieff, 1989). Brain concentrations are several fold higher than those in the blood in both exposed (i.e., harmane-injected) laboratory animals and in control animals (Anderson et al., 2006; Zetler et al., 1972). Although harmane is produced endogenously, it is also present in the diet (especially in meats but also in other foods) and exogenous exposure is thought to be the main source of the body's harmane (Pfau and Skog, 2004).

We had hypothesized that this neurotoxin could play a role in the etiology of ET, and in 2002, we demonstrated that blood harmane concentration was elevated in an initial sample of 100 ET patients compared with 100 controls (Louis et al., 2002b). During the past five years, we have assembled a replicate sample of 150 new ET cases and 135 new controls. We assessed blood harmane concentrations in this new sample of cases vs. controls and compared these concentrations in familial with non-familial ET cases, and we now report these results.

Methods

Participants

Recruitment began in 2002 and has continued to present (October 2007). All ET cases were enrolled in a study of the environmental epidemiology of ET at Columbia-University Medical Center (CUMC). By design, ET cases were identified from one of five sources: (1) a computerized billing database at the Neurological Institute of New York, CUMC (N = 67), (2) a computerized billing database in the Neurology Department at the Weill Medical College of Cornell University (N = 6), (3) advertisements to members of the International Essential Tremor Foundation (N = 59), (4) ET cases identified in a population-based study of aging in northern Manhattan (N = 4) (Louis et al., 2005), and (5) word of mouth (e.g., ET cases identified above who had friends with ET)(N = 14). All cases had received a diagnosis of ET from their treating neurologist (N = 146) or study neurologist (N = 4) and all lived within two hours driving distance of CUMC in New York, New Jersey, and Connecticut. The case finding process included examining the office records of all identified potential patients; patients with diagnoses or physical signs of dystonia, Parkinson's disease (PD), or spinocerebellar ataxia were excluded.

Control subjects were recruited during the same time period. Controls were identified using random digit telephone dialing within a defined set of telephone area codes that were

represented by the ET cases (e.g., 212, 201, 203, 516, 718, 914) within the New York Metropolitan area. Controls were frequency-matched to cases based on gender, race (White, African-American, Hispanic, Asian, Other), and current age (5 year intervals). The CUMC Internal Review Board approved of all study procedures and written informed consent was obtained at the time of enrollment.

ET cases and controls were screened for cognitive impairment using the 10-minute Telephone Interview for Cognitive Status (Brandt J, 1988). This was done to minimize the enrollment of individuals with invalid medical or occupational histories. Eight individuals (one case and seven controls) with cognitive impairment (score < 30 of 41) were excluded.

One hundred-fifty (83.3%) of the 180 potential cases and 135 (59.7%) of the 226 potential controls who were contacted agreed to participate. Participants (enrollees) differed from non-participants in terms of age (65.4 ± 14.9 years vs. 71.3 ± 9.6 years, t = 4.01, p < 0.001) and gender (53.0% vs. 66.1% women, X^2 = 5.97, p = 0.02).

Clinical Evaluation

All case and control subjects were evaluated in person by a trained tester who administered clinical questionnaires and performed a videotaped examination. Most evaluations were home visits, and therefore were performed in the late morning or early afternoon, making fasting blood harmane concentrations impractical. Data suggest that plasma concentrations of harmane do not change significantly during the day (Rommelspacher et al., 1991). In one study (Rommelspacher et al., 1991), human subjects ingested food or ethanol, and plasma harmane concentrations were measured hourly for eight hours. The concentration remained stable. The same investigators also demonstrated that variability in concentration was minimal over a longer (three week) period (Rommelspacher et al., 1991). Our own data indicate that log blood harmane concentration is not correlated with the time latency since last food consumption (r = -0.097, p = 0.49 [N = 52]).

The tester collected demographic information (age in years, gender, years of education, race [non-Hispanic white vs. others]), clinical information (tremor duration in years), and family history information using a structured questionnaire. ET cases and controls were classified as having a family history of ET if they reported at least one first-degree relative with ET. Subjects were classified as current ethanol users if they drank at least one alcoholic beverage (beer, wine, other) per month. Current smoking status was assessed in each subject, as was past cigarette use, which allowed us to calculate cigarette pack-years. Medical co-morbidity was assessed with the Cumulative Illness Rating Scale, in which the severity of medical problems (0 [none] - 3 [severe]) was rated in 14 bodily systems (e.g., cardiac, hepatic) and a Cumulative Illness Rating Scale score was assigned (range = 0 - 42) to each subject (Linn et al., 1968). The names of all current medications were recorded separately and then collapsed into 19 classes of medications (e.g., neuroleptic medications, cardiac medications, anti-cancer medications, narcotics, medications for ET, selective serotonin reuptake inhibitors, oral hypoglycemic agents, etc).

Weight and height were assessed using a balance scale designed for field surveys (Scale-Tronix 5600, White Plains, NY) and a movable anthropometer (GPM Martin Type, Pfister Inc, Carlstadt, NJ). Body mass index was calculated as weight in kg divided by the square of height in meters.

The tester videotaped a tremor examination in all subjects (Louis et al., 1997; Louis et al., 2002b), and each of 12 videotaped action tremor items was rated by Dr. Louis on a scale from 0 to 3, resulting in a total tremor score (range = 0 - 36 [maximum]). The diagnosis of ET was confirmed by Dr. Louis using published diagnostic criteria (moderate or greater amplitude

tremor during three activities or a head tremor in the absence of PD, dystonia or another neurological disorder)(Louis et al., 1998a; Louis et al., 1997; Louis et al., 2002b). None of the cases or control subjects had PD or dystonia.

Blood Harmane Concentrations

During the clinical evaluation, phlebotomy was performed. Blood concentrations of harmane were measured blinded to any clinical information, including age, gender, and diagnosis. Harmane concentrations in blood were quantified by a well-established high performance liquid chromatography (HPLC) method in this group and used in our previous studies (Zheng et al., 2000). In short, one volume (9 - 12 mL) of whole blood was mixed with half-a-volume (5 - 6 mL) of 1 M NaOH. Following vortex for 30 sec., the samples were placed on a horizontal rotator and shaken at room temperature for 30 min. An aliquot (15 mL) of the extraction solution consisting of ethyl acetate and methyl-*t*-butyl ether (2:98, V:V) was added to the tube. The tube was then vigorously shaken by hand for 1-2 min, followed by shaking on a horizontal rotor at room temperature for 45 mins. After centrifugation at 3000 x g for 10 min, the upper organic phase was separated. The extraction procedure was repeated two additional times. The organic phase was combined and evaporated under nitrogen to dryness. The samples were reconstructed in 0.25 mL of methanol. After centrifugation at 3000 x g for 10 min, the supernatant was transferred to autosampler vials with sealed caps for HPLC analysis.

A Waters Model 2695XE complete HPLC system including autosampler, temperature control module, seal wash and degasser, and a Waters Model 2475 Multi-channel fluorescent detector was used for separation and quantification. Separation was accomplished using an ion-interaction, reversed-phase Econosphere C_{18} column (ODS2, 5 μm, 250 × 4.6 mm) attached to a Spherisorb guard column (ODS2, 5 μm, 10 × 4.6 mm). Both analytical and guard columns were purchased from Alltech (Deerfield, IL). An isocratic mobile phase consisted of 17.5 mM potassium phosphate buffer, pH 6.5 (equal molar concentration of both monobasic and dibasic potassium salts) and methanol (30:70, V:V). A 50-μL aliquot of sample extracts was injected and the separation performed at room temperature at a flow rate of 1 mL/min. The detector was set at an excitation wavelength of 300 nm and an emission wavelength of 435 nm. A Dell Window based computer equipped with Waters data analysis package was used to collect and analyze the data. The identity of harmane on HPLC chromatographs previously has been clarified (Guan et al., 2001; Zheng et al., 2000). The intraday precision, measured as a coefficient of variation at 25 ng/mL, was 6.7% for harmane. The interday precision was 7.3% for harmane (Zheng et al., 2000).

Statistical Analyses

The empirical distribution of harmane is positively skewed. Using a one-sample Kolmogorov-Smirnov test, we tested whether harmane concentration was normally distributed and it was not (Kolmogorov-Smirnov test, z = 7.43, p < 0.001). Therefore, harmane concentrations were logarithmically transformed. Case-control differences in log blood harmane concentrations were assessed using Student's t tests. In confirmatory analyses, a non-parametric (Mann Whitney U) test also was performed on harmane data that were not logarithmically transformed. Log blood harmane concentrations were also stratified as above the median and below the median and also stratified into tertiles. The total tremor score was also stratified into high score (≥ 25) and low score categories. Chi-square (χ^2) tests were used to analyze proportions, and Student's t tests and analysis of variance (ANOVA) were used to examine group differences in continuous variables. Pearson (r) correlation coefficients were used to assess correlations between continuous variables. To assess the null hypothesis that blood harmane concentration was not a predictor of diagnostic group (ET vs. control), logistic regression analysis was performed using diagnostic group as the outcome, and log blood harmane concentration as the primary independent variable. We repeated this analysis using log blood harmane

concentration tertiles as the independent variables and calculated odds ratios (OR) with 95% CI, comparing the second and third tertile to the lowest tertile. We considered a number of potential confounders (age in years, gender, race, years of education, body mass index, Cumulative Illness Rating scale score, current cigarette smoker, cigarette pack years, current ethanol user, and medications [19 classes]) and included these in the adjusted logistic regression analyses if they were associated with either ET or blood harmane concentration in this dataset or prior publications (Louis et al., 2002b). In some analyses, we tested for trend by treating log blood harmane concentration as the dependent variable in a linear regression analysis and examining the association with an ordinally-distributed independent variable (e.g., ET cases with a family history of ET, ET cases without a family history of ET, controls). Statistical analyses were performed in SPSS (Version 13.0).

Results

The 150 ET cases and 135 controls were similar in terms of age, gender, and other demographic and clinical features (Table). As expected, cases had a higher mean $\pm$ SD total tremor score than controls (18.1 $\pm$ 6.7 vs. 3.9 $\pm$ 2.8, t = 22.7, p <0.001). Mean disease duration (cases) was 23.0 +/- 18.8 years and 61 (40.7%) were taking a medication to treat ET.

Using our control sample, we examined the correlates of log blood harmane concentration. Log blood harmane concentration was not associated with age in years (r = -0.001, p = 0.99), years of education (r = 0.02, p = 0.82), number of rooms in home (r = 0.03, p = 0.71), body mass index (r = -0.02, p = 0.85) or Cumulative Illness Rating Score (r = -0.008, p = 0.93). Log blood harmane concentration did not differ by gender (0.42 $\pm$ 0.69 g $^{-10}$/ml for men and 0.31 $\pm$ 0.57 g $^{-10}$/ml for women, t = 0.98, p = 0.33) or by race (0.35 $\pm$ 0.63 g $^{-10}$/ml for non-Hispanic whites and 0.36 $\pm$ 0.59 g $^{-10}$/ml for others, t = 0.05, p = 0.96). There was no difference between current cigarette smokers and nonsmokers (0.34 $\pm$ 0.43 g $^{-10}$/ml and 0.36 $\pm$ 0.64 g $^{-10}$/ml, respectively, t = 0.11, p = 0.91) and, among current smokers, no association with number of pack years (r = -0.14, p = 0.76). Log blood harmane concentration was similar in subjects who were vs. were not current ethanol users (0.33 $\pm$ 0.81 g $^{-10}$/ml vs. 0.42 $\pm$ 0.71 g $^{-10}$/ml, t = 0.47, p = 0.64). Log blood harmane concentration was not associated with current use of any of the 19 classes of medications.

Mean log blood harmane concentration was nearly 50% higher in cases than controls (0.50 $\pm$ 0.54 g $^{-10}$/ml vs. 0.35 $\pm$ 0.62 g^{-10}/ml, t = 2.09, p = 0.038)(Figure 1). A non-parametric test (Mann Whitney U) on non-transformed data (median harmane = 2.61 g $^{-10}$/ml in cases and 1.82 g $^{-10}$/ml in controls) confirmed this difference (z = 2.41, p = 0.016). Strata were created based on the median log blood harmane concentration (0.3811 g $^{-10}$/ml); 55% of cases vs. 42% of controls had a high log blood harmane concentration based on this median split (X^2 = 4.40, p = 0.036). Log blood harmane concentration was similar in ET cases from each of the five sources (ANOVA F = 0.32, p = 0.86).

In an unadjusted logistic regression analysis, log blood harmane concentration was associated with the outcome (diagnosis of ET vs. normal)(OR = 1.56, 95% CI = 1.02 - 2.39, p = 0.04) (i.e., for every doubling of the harmane concentration, the odds of ET increased by 56%). In logistic regression analyses that adjusted for each one of the following variables individually and in then in combination, the association remained unchanged: age in years, gender, non-Hispanic white race, years of education, current smoker (e.g., in a model that adjusted for each of these variables in combination, OR = 1.56, 95% CI = 1.01 - 2.42, p = 0.04). In an unadjusted logistic regression analysis, odds of ET was 1.90 (95% CI = 1.07 - 3.39, p = 0.029) in the highest log blood harmane tertile and 1.55 (95% CI = 0.88 - 2.73, p = 0.13) in the middle log blood harmane tertile (reference group = lowest log blood harmane tertile). In a logistic regression analysis that adjusted for age in years, gender, non-Hispanic white race, years of

education, current smoker, the odds of ET was 1.97 (95% CI = 1.09 - 3.54, p = 0.02) in the highest log blood harmane tertile and 1.70 (95% CI = 0.95 - 3.03, p = 0.07) in the middle log blood harmane tertile (reference group = lowest log blood harmane tertile).

Among ET cases, there was no correlation between log blood harmane concentration and total tremor score (Pearson's r = -0.01, p = 0.87), yet in a small subgroup of 24 ET cases with high total tremor scores (total tremor score ≥ 25), the mean log blood harmane concentration was 0.55 ± 0.54 g $^{-10}$/ml. To test for trend, we performed a linear regression analysis in which log blood harmane concentration was the outcome variable and the ordinal independent variable was coded as follows: ET with high total tremor score (2), remainder of ET cases (1), and controls (0), and beta = 0.16, p = 0.03, indicating a trend. Tremor duration was not correlated with log blood harmane concentration (r = -0.02, p = 0.84). We also stratified the ET cases by family history. The 89 ET cases with a family history of ET had the highest log blood harmane concentration (0.53 ± 0.57 g $^{-10}$/ml), followed by ET cases without a family history of ET (0.43 ± 0.45 g $^{-10}$/ml), and then finally the controls, who had the lowest concentration (0.35 ± 0.62 g^{-10}/ml) (test for trend [linear regression analysis] beta = 0.09, p = 0.026, Figure 2). Among controls, the 17 (12.6%) with a family history of ET were similar to the 118 (87.4%) without a family history of ET in terms of log blood harmane concentration (0.30 ± 0.46 g $^{-10}$/ml vs. 0.36 ± 0.65 g $^{-10}$/ml, t = 0.40, p = 0.69).

Discussion

Blood harmane concentration was elevated in ET cases compared to control subjects. The higher concentration in familial ET cases suggests that the mechanism for this elevated concentration may be at least partly genetic.

Both genetic and environmental factors are likely to play a role in the etiology of ET. Many kindreds with autosomal dominant inheritance of ET have been described, and linkage has been demonstrated to regions on chromosomes 2p, 3q, and 6p, although at present the genes that are responsible for ET genes have not been identified (Deng et al., 2007; Gulcher et al., 1997; Higgins et al., 1997; Shatunov et al., 2006). In twin studies, concordance in monozygotic twins was 60 - 63%; these and other data indicate that non-genetic (environmental) factors also play an important role in disease etiology (Lorenz et al., 2004; Louis, 2001; Tanner et al., 2001). Environmental factors are similarly thought to play a significant role in several other late life neurological diseases, including PD and Alzheimer's disease (Baldereschi et al., 2007; Dick, 2006; Gorell et al., 1997; Gorell et al., 1999; Gorell et al., 1998; Perl, 1985; Racette et al., 2001; Ritz and Yu, 2000; Rybicki et al., 1993; Semchuk et al., 1992; Shcherbatykh and Carpenter, 2007). The contribution of environmental risk factors to disease etiology has been examined in detail in epidemiological studies of PD, Alzheimer's disease, amyotrophic lateral sclerosis (Baldereschi et al., 2007; Dick, 2006; Gorell et al., 1997; Gorell et al., 1999; Gorell et al., 1998; Morahan et al., 2007; Perl, 1985; Racette et al., 2001; Ritz and Yu, 2000; Rybicki et al., 1993; Semchuk et al., 1992; Shcherbatykh and Carpenter, 2007). The identification of toxins such as 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) and pesticides as precipitants of PD, has greatly advanced our understanding of putative disease mechanisms; for example, the MPTP model has become the most commonly used experimental model for PD (Langston et al., 1984). The study of such toxins in ET lags far behind. It is commonly stated that 50% of ET cases are non-familial (Lambert and Waters, 1999; Louis and Ottman, 1996). Given a population prevalence for ET of 4.0% after age 39 years (Dogu et al., 2003), this suggests that approximately 2.0% of the population aged ≥ 40 years has a nonfamilial form of ET, yet the environmental correlates for this tremor are only just beginning to be explored.

In the selection of possible toxic environmental causes of ET for investigation, β-carboline alkaloids such as harmane are an obvious choice. These toxins have a structural similarity to

the neurotoxin MPTP, which has served as one of the main animal models for PD (Langston et al., 1984; Smeyne and Jackson-Lewis, 2005). Like MPTP, β-carboline alkaloids are highly neurotoxic, and it has been known for approximately 100 years that administration of β-carboline alkaloids to a wide variety of laboratory animals produces severe action tremor that resembles ET (Du et al., 1997). Acute exposure to β-carboline alkaloids results in an intense and generalized action tremor in a broad range of laboratory species including mice, cats, and monkeys. β-carboline alkaloid administration is the main animal model for ET and new pharmacotherapies are tested using exposed animals (Handforth and Krahl, 2001; Krahl et al., 2004; Martin and Handforth, 2006; Martin et al., 2005; Sinton et al., 1989). The β-carboline alkaloid tremor shares many features with ET. These include several of the principal clinical features (e.g., tremor frequency and electromyographic characteristics of the tremor) and drug-response characteristics (pharmacological responsiveness to benzodiazepines, alcohol, and barbiturates)(Cross et al., 1993; Fuentes and Longo, 1971; Milner et al., 1995; Rappaport et al., 1984; Sinton et al., 1989; Trouvin et al., 1987). Also, underlying brain changes are similar. Changes, including neuronal loss, have been shown to occur in the ET cerebellum (Axelrad JE, 2007 In Press; Louis et al., 2002a; Louis et al., 2004; Louis et al., 2006a; Louis et al., 2006b; Pagan et al., 2003; Shill H, 2007). Similarly, β-carboline alkaloids produce toxic damage with significant loss of cerebellar Purkinje cells (Du et al., 1997; Milner et al., 1995; O'Hearn et al., 1993; O'Hearn and Molliver, 1993; O'Hearn and Molliver, 1997; Robertson, 1980; Sinton et al., 1989). While β-carboline alkaloids are produced endogenously in the human body (Gearhart et al., 2000; Wakabayashi et al., 1997), one study estimated that dietary sources were fifty times greater than endogenous sources (Pfau and Skog, 2004). β-carboline alkaloids are primarily found in muscle foods (beef, chicken, pork, fish) at ng/g concentrations and cooking leads to increased concentrations (Gross et al., 1993; Layton et al., 1995; Skog, 1993; Skog et al., 1998). The formation of β-carboline alkaloids in cooked meat is a function of cooking temperature and time, with concentrations increasing most rapidly with time at higher temperatures (Sinha et al., 1998a; Sinha et al., 1998b). Pan frying and grill/barbequing produce the highest concentrations of β-carboline alkaloids while oven cooking and microwaving produce no or negligible increases in β-carboline alkaloid concentrations. In addition to their high concentration in meat, β-carboline alkaloids are also present in small concentrations in some plants, including tobacco and coffee (Herraiz, 2004). They are also present in ethanol and other foods. The effect of chronic, low-level β-carboline alkaloid exposure is not known.

This study did not intend, nor did it provide an explanation for the case-control difference. The latter deserves further study. Nonetheless, possibilities include an increased exogenous exposure to these chemicals in ET cases through diet, increased endogenous production in ET cases, impaired metabolism in ET cases, or a combination of these factors (e.g., increased dietary exposure and a genetic susceptibility to impaired metabolism). The higher concentration in familial ET cases, which is something we did not find in our earlier study (Louis et al., 2002b), suggests that the mechanism may involve genetic factors.

This study had limitations. We did not assess fasting blood harmane concentrations. Therefore, we cannot assess the extent to which the case-control difference reflects a difference in dietary intake of harmane. However, our data indicate that log blood harmane concentration is not a function of time since last food consumption (r = -0.097, p = 0.49) and other investigators have demonstrated stable blood concentrations following food ingestion (Rommelspacher et al., 1991). Also, we did not assess liver function, variability in the cytochrome P450 system, or renal function to see whether factors that might influence the metabolism of harmane differed between cases and controls. Also, participants differed from non-participants in terms of age and gender. However, in our analyses, neither age nor gender was associated with log blood harmane concentration, so that this is unlikely to have influenced our results. The strengths of the study include the uniqueness of the question (there are no other studies, other than our

original publication examining this issue)(Louis et al., 2002b), the large sample size, the careful attempt to match the cases and controls, and the ability to adjust for multiple potential confounding factors.

It would be important to study the underlying mechanisms for this elevated blood harmane concentration, and such studies are currently underway. In the future, it would also be important to try to reproduce our finding in additional samples of ET cases ascertained in different settings (e.g., ET cases ascertained in a population-based setting or additional samples of ET cases ascertained from other geographic regions). Prospective studies could address whether this elevated blood harmane concentration precedes the diagnosis or follows the diagnosis of ET.

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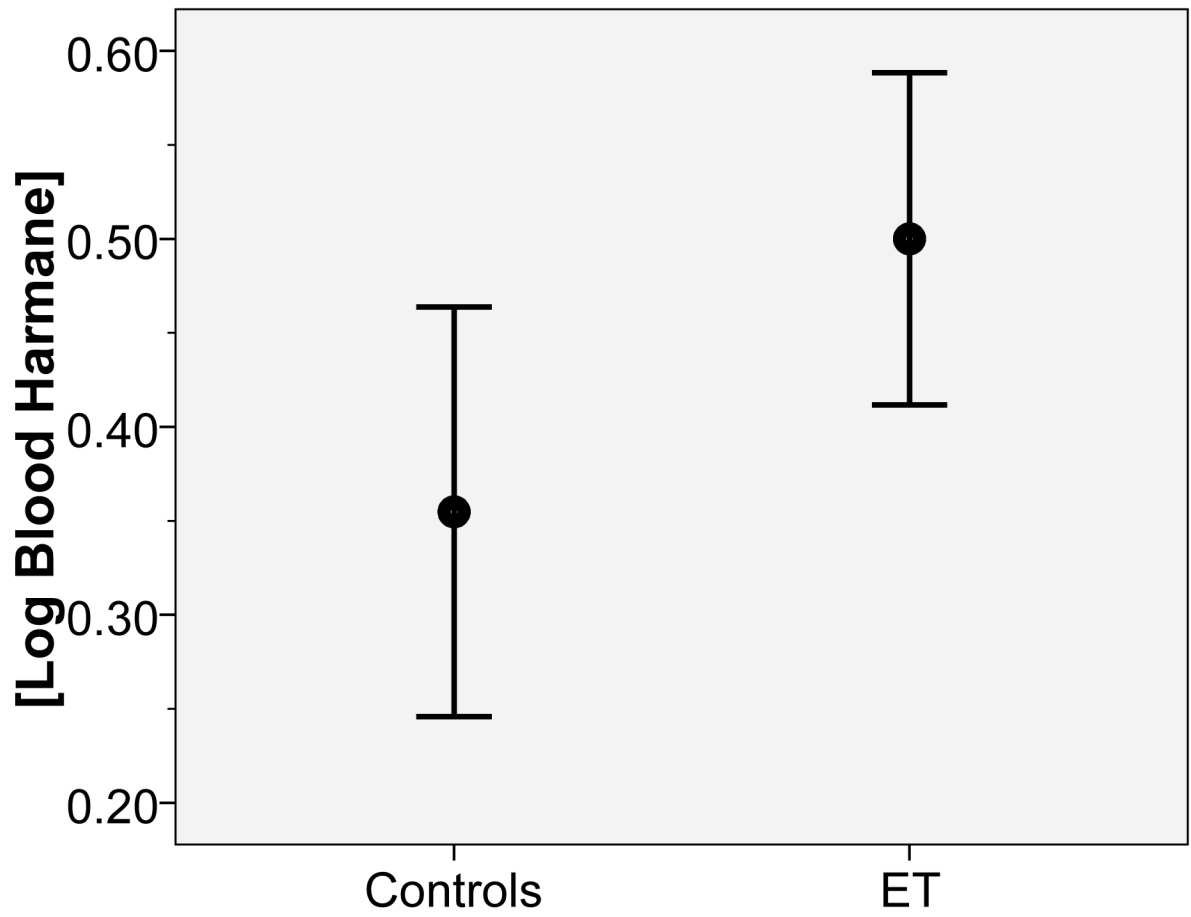
Figure 1.
Log blood harmane concentration in controls and ET cases (p = 0.038). The circles represent means and bars represent 2 standard errors.
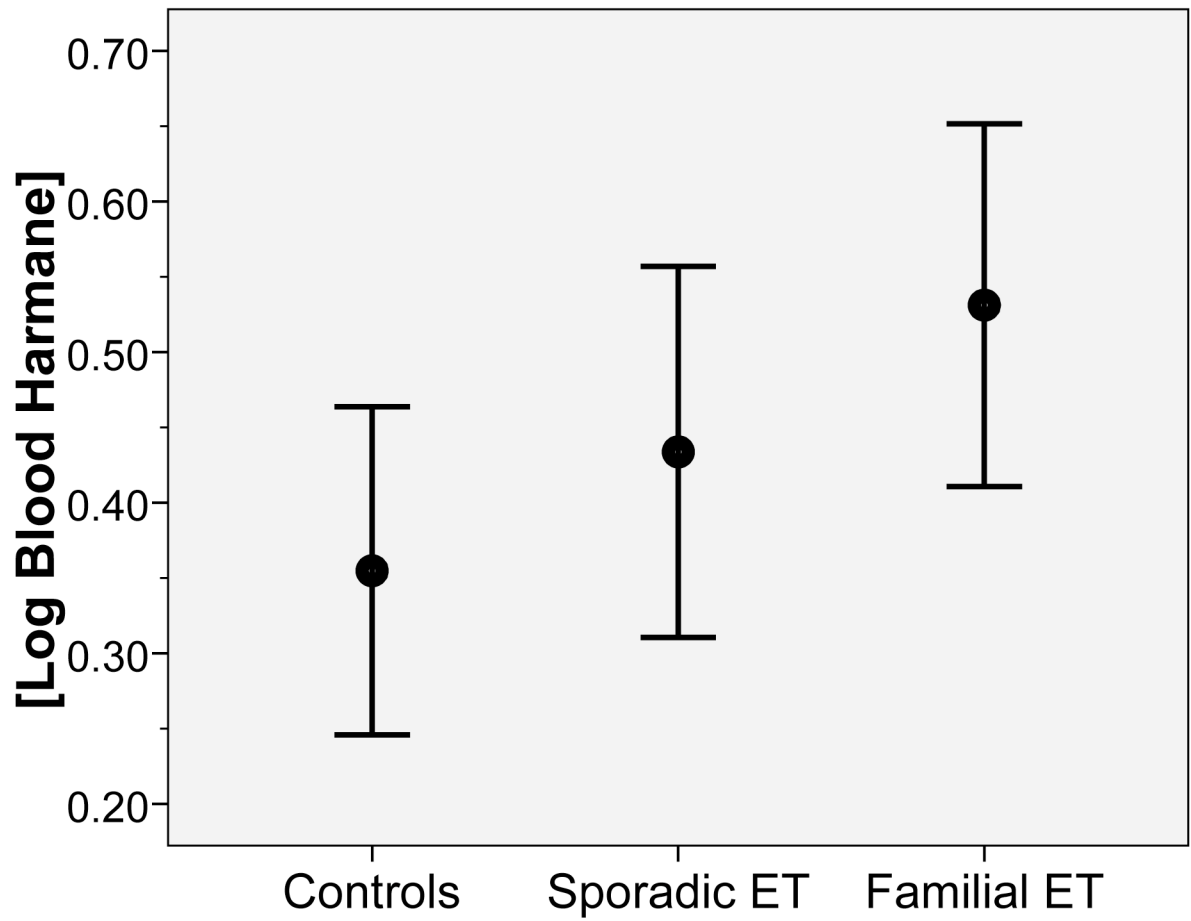
Figure 2.
Log blood harmane concentration in controls, sporadic ET and familial ET. The circles represent means and bars represent 2 standard errors. Test for trend (linear regression analysis) beta = 0.09, p = 0.026.

Table

Characteristics of ET cases vs. controls

Characteristic	ET Cases (N = 150)	Controls (N = 135)	Significance
Age in years	65.3 ± 15.5	65.5 ± 14.2	t = 0.07, p = 0.94
Female gender	75 (50.0)	76 (56.3)	X^2 = 1.13, p = 0.29
Non-Hispanic white race	140 (93.3)	119 (88.1)	X^2 = 3.19, p = 0.36
Years of education	15.5 ± 3.9	15.3 ± 3.6	t = 0.40, p = 0.69
Number of rooms in home	5.8 ± 2.5	5.6 ± 3.5	t = 0.63, p = 0.53
Body mass index kg/m^2	27.4 ± 12.2	27.9 ± 6.0	t = 0.38, p = 0.70
Current cigarette smoker	14 (9.4)	11 (8.1)	X^2 = 0.14, p = 0.71
Pack-years (among current cigarette smokers)	25.3 ± 24.2	43.4 ± 25.7	t = 1.44, p = 0.17
Cumulative Illness Rating Scale score	6.2 ± 4.1	5.7 ± 4.2	t = 0.99, p = 0.32

Values are mean ± SD or numbers (percentages).