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Relationship between blood harmane and harmine concentrations in familial essential tremor, sporadic essential tremor and controls

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ABSTRACT

Introduction: Harmane, a potent tremor-producing β-carboline alkaloid, may play a role in the etiology of essential tremor (ET). Blood harmane concentrations are elevated in ET cases compared with controls yet the basis for this elevation remains unknown. Decreased metabolic conversion (harmane to harmine) is one possible explanation. Using a sample of >500 individuals, we hypothesized that defective metabolic conversion of harmane to harmine might underlie the observed elevated harmane concentration in ET, and therefore expected to find a higher harmane to harmine ratio in familial ET than in sporadic ET or controls.

Methods: Blood harmane and harmine concentrations were quantified by high performance liquid chromatography.

Results: There were 78 familial ET cases, 187 sporadic ET cases, and 276 controls. Blood harmane and harmine concentrations were correlated with one another (Spearman's $r = 0.24$, $p < 0.001$). The mean (±SD) harmane/harmine ratio = 23.4 ± 90.9 (range = 0.1–987.5). The harmane/harmine ratio was highest in familial ET (46.7 ± 140.4), intermediate in sporadic ET (28.3 ± 108.1), and lowest in controls (13.5 ± 50.3) ($p = 0.03$). In familial ET cases, there was no association between this ratio and tremor severity (Spearman's $r = 0.08$, $p = 0.48$) or tremor duration (Spearman's $r = 0.14$, $p = 0.24$).

Conclusion: The basis for the elevated blood harmane concentration, particularly in familial ET, is not known, although the current findings (highest harmane/harmine ratio in familial ET cases) lends support to the possibility that it could be the result of a genetically-driven reduction in harmane metabolism.

1. Introduction

Essential tremor (ET), a neurological disease whose cardinal feature is action tremor of the arms, is widespread and it affects all age groups. With a prevalence of 4.0% in individuals aged ≥40 years and 8.7% in individuals in their ninth decade (Dogu et al., 2003; Benito-Leon et al., 2003), it is one of the most common neurological diseases (Louis et al., 2009; Louis and Ferreira, 2010). In addition to action tremor, patients may exhibit a range of other neurological signs, including cognitive impairment (Benito-Leon et al., 2006), gait ataxia and incoordination (Singer et al., 1994). Both genetic (Higgins et al., 1997; Gulcher et al., 1997; Clark et al., 2010; Shatunov et al., 2006) and non-genetic (i.e., environmental) factors (Jiménez-Jiménez et al., 2007; Salemi et al., 1998; Louis, 2001) are likely to play a role in disease etiology.

The β-carboline alkaloids are a group of neurotoxic chemicals that produce action tremor. Laboratory animals injected with high doses develop an acute action tremor that clinically resembles that seen in patients with ET (Fuentes and Longo, 1971; Zetler et al., 1972). Human volunteers who have been exposed to high doses may display a coarse, reversible action tremor (Lewin, 1928).

Harmane (1-methyl-9H-pyrido[3,4-β]indole) is among the most potent tremor-producing β-carboline alkaloids; subcutaneously administered harmane (38 mg/kg) produces tremor in mice (McKenna, 1996). With its high lipid solubility (Zetler et al., 1972), harmane is readily distributed to and within the brain (Moncrieff, 1989; Anderson et al., 2006; Matsubara et al., 1993). Brain concentrations are several fold higher than those in the blood in both exposed (i.e., harmane-injected) laboratory animals as well as control animals (Zetler et al., 1972; Anderson et al., 2006). Although harmane is produced endogenously, it is also present in

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the diet; exogenous exposure is thought to be the primary source of bodily harmane (Pfau and Skog, 2004).

Hypothesizing that this neurotoxin could play an etiological role in ET, a disease for which no environmental toxins had as yet been identified, in 2002 we demonstrated that blood harmane concentration was elevated in an initial sample of 100 ET cases compared with 100 controls (Louis et al., 2002). In a replicate sample several years later (150 new ET cases and 135 new controls), we demonstrated a similar elevation; furthermore, we reported that concentrations were particularly high among familial ET cases (Louis et al., 2008a).

The basis for the elevated blood harmane concentration in ET is not known, although possibilities include increased dietary consumption and decreased metabolic turnover. An initial study of diet reported only weak evidence for dietary differences (i.e., slightly increased meat consumption) between male but not female ET cases and controls (Louis et al., 2008b). We previously demonstrated that blood harmane concentration was highest in familial ET cases, providing support for the notion that genetic/metabolic factors are of mechanistic importance (Louis et al., 2008a). The metabolic pathway for harmane is not fully known, although it is probable that it is converted by the liver cytochrome P-450 system to harmine (7-methoxy-1-methyl-9H-pyrido[3,4-β]-indole) through a simple hydroxylation and then methylation step (Guan et al., 2001).

To further explore the genetic/metabolic hypothesis, we examined data on blood concentrations of harmane and harmine in more than 500 individuals, comprised of three groups: familial ET, sporadic ET and controls. We hypothesized that defective metabolic conversion of harmane to harmine might underlie the observed elevated harmane concentration in ET, and therefore expected to find a higher harmane to harmine ratio in familial ET than in sporadic ET or controls.

2. Methods

2.1. Participants

Recruitment began in 2000 and continued to 2009. 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 several sources, with the major ones being a computerized billing database of ET patients at the Neurological Institute of New York (CUMC) and advertisements to members of the International Essential Tremor Foundation (Louis et al., 2002, 2008a). All cases had received a diagnosis of ET from their treating neurologist and lived within 2 h driving distance of CUMC in New York, New Jersey, and Connecticut.

Control subjects were recruited during the same time period (Louis et al., 2002, 2008a). 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, and current age. The CUMC Internal Review Board approved of all study procedures and signed, written informed consent was obtained at the time of enrollment (Louis et al., 2002, 2008a).

We excluded 134 participants who did not have phlebotomy (refusals, unsuccessful attempts); these 134 were similar to the remaining 541 participants in terms of diagnosis (22 [16.4%] vs. 78 [14.4%] familial ET, $p = 0.56$), gender (72 [53.7%] vs. 292 [54.0%] women, $p = 0.96$), education (15.1 ± 3.3 vs. 15.4 ± 3.5 years, $p = 0.37$), and number of rooms in their home (a socioeconomic variable) (5.8 ± 3.1 vs. 5.7 ± 2.7, $p = 0.71$). The 134 participants who were excluded were, on average, 5.3 years older than the remaining 541 participants (71.5 ± 12.7 vs. 66.2 ± 14.3 years, $p < 0.001$).

2.2. Clinical evaluation

All cases and controls 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. Our prior data indicate that blood harmane concentration is not correlated with the time latency since last food consumption ($r = -0.097$, $p = 0.49$ [$N = 52$]) (Louis et al., 2008a). Other published 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 8 h. The concentration remained stable. The same investigators also demonstrated that variability in concentration was minimal over a longer (i.e., 3-week) period (Rommelspacher et al., 1991).

The tester used a structured questionnaire to collect demographic information (age in years, gender, years of education, race [non-Hispanic white vs. others], number of rooms in home), tremor characteristics (e.g., duration and age of onset), medication use, and family history information. ET cases were classified as having a familial ET if they reported at least one first-degree relative with ET; otherwise they were classified as sporadic ET. Current smoking status was assessed in each participant. 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 participant (Linn et al., 1968). Data were also collected on ethanol intake and converted into number of grams of ethanol consumed per day. In the final months of 2006, data collection began on the time latency between last ingestion of food or beverages and time of phlebotomy.

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 participants (Louis et al., 2002, 1997). Each of 12 videotaped action tremor items was rated by Dr. Louis on a scale from 0 to 3, a total tremor score was assigned (range = 0–36), and the diagnosis of ET was confirmed by Dr. Louis using published diagnostic criteria (moderate or greater amplitude tremor during $\geq$3 activities or a head tremor in the absence of Parkinson's disease [PD], dystonia or another neurological disease) (Louis et al., 2002, 1997, 1998). None of the cases or control subjects had PD or dystonia.

2.3. Blood harmane concentrations

At the time of the clinical evaluation, phlebotomy was performed. When the evaluation was performed in the participant's home, blood tubes were temporarily stored on ice packs and then several hours later transferred to a −20 °C freezer; if performed at CUMC, they were placed immediately into a −20 °C freezer. Blood harmane and harmine concentrations were measured blinded to any clinical information with a well-established high performance liquid chromatography (HPLC) method used in our previous studies (Louis et al., 2002, 2008a, 2005a; 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 s, 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 min. After centrifugation at 3000 × *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 × *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 mm × 4.6 mm) attached to a Spherisorb guard column (ODS2, 5 μm, 10 mm × 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 and harmine 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 and 3.4% for harmine. The interday precision was 7.3% for harmane and 5.4% for harmine (Zheng et al., 2000).

2.4. Statistical analyses

Chi-square tests were used to analyze proportions, and Student's *t*-tests were used to examine group differences in continuous variables.

The empirical distribution of blood harmane and harmine concentration were positively skewed (one-sample Kolmogorov–Smirnov test, $z = 9.47$ and 9.88, both $p < 0.001$). Hence, non-parametric tests (Mann–Whitney *U*, Kruskal–Wallis, Spearman's rho) were used when assessing these variables.

HA/HI ratio was the blood harmane concentration divided by the blood harmine concentration. To assess the null hypothesis that HA/HI ratio did not differ by diagnostic group (familial ET vs. sporadic ET vs. controls), a Kruskal–Wallis test was performed. We considered a number of potential confounders (age in years, gender, race, years of education, number of rooms in home, body mass index, Cumulative Illness Rating scale score, current cigarette smoker). If any of these were associated with ET or blood harmane concentration in either univariate analyses or in prior publications (Louis et al., 2002, 2008a), we performed stratified analyses, assessing whether the observed difference in HA/HI ratio remained stable within different strata. Due to the loss of power in these stratified analyses, *p* values were not reported; rather, the aim of these analyses was to determine whether the magnitude of the diagnostic group difference persisted after stratification. Statistical analyses were performed in SPSS (Version 17.0).

3. Results

There were 541 participants, including 265 ET cases (78 familial ET and 187 sporadic ET) and 276 controls. Familial ET cases were similar to controls with respect to all variables except body mass index and race (Table 1); sporadic ET cases differed from controls with respect to gender and race (Table 1). Mean disease duration (ET cases) was 24.9 ± 20.2 years, age of tremor onset (ET cases) was 42.4 ± 23.6 years, and 140 (52.8%) were taking a medication to treat ET.

Using our control sample, we examined the correlates of the HA/HI ratio. HA/HI ratio was associated with body mass index (Spearman's $r = 0.21$, $p < 0.001$) and modestly with number of rooms in home (Spearman's $r = 0.14$, $p = 0.02$). HA/HI ratio was not associated with age in years (Spearman's $r = 0.05$, $p = 0.40$), years of education (Spearman's $r = -0.05$, $p = 0.45$) or Cumulative Illness Rating Scale score (Spearman's $r = 0.08$, $p = 0.20$). HA/HI ratio did not differ by gender (19.3 ± 72.5 for men and 9.4 ± 25.0 for women, Mann–Whitney $z = 0.39$, $p = 0.70$) or by race (14.1 ± 53.8 for non-Hispanic whites and 9.4 ± 18.4 for others, Mann–Whitney $z = 1.02$, $p = 0.31$). There was no difference between current cigarette smokers and nonsmokers (19.7 ± 77.4 and 12.9 ± 47.1, Mann–Whitney $z = 0.46$, $p = 0.64$). There were 61 controls whose data were available on the time latency between last ingestion of food or beverages and time of phlebotomy. There was no association between this latency and blood harmane concentration (Spearman's $r = 0.12$, $p = 0.34$) or HA/HI ratio (Spearman's $r = 0.11$, $p = 0.41$).

The blood harmane and harmine concentrations were significantly correlated with one another (Spearman's $r = 0.24$, $p < 0.001$); the correlation was present for all three diagnostic groups ($r = 0.27$, $p < 0.001$ [controls]; $r = 0.19$, $p = 0.01$ [sporadic ET]; $r = 0.27$, $p = 0.02$ [familial ET]).

The mean ± SD HA/HI ratio = 23.4 ± 90.9 (range = 0.1–987.5). The HA/HI ratio was highest in familial ET (46.7 ± 140.4), intermediate in sporadic ET (28.3 ± 108.1), and lowest in controls (13.5 ± 50.3) (Kruskal–Wallis $p = 0.03$) (Fig. 1). We assessed the possible confounding effects of age, gender, race, body mass index and number of rooms in home on the association between diagnosis and HA/HI ratio (Table 2). Within nearly all strata, the HA/HI ratio was highest in familial ET, intermediate in sporadic ET and lowest in controls

Table 1
Characteristics of 265 ET cases vs. 276 controls.

Characteristic	ET cases (*N* = 265)		Controls (*N* = 276)
	Familial ET (*N* = 78)	Sporadic ET (*N* = 187)	
Age in years	65.9 ± 18.0	66.1 ± 14.5	66.3 ± 12.9
Female gender	42 (53.8)	87 (46.5)**	163 (59.1)
Non-Hispanic white race	74 (94.9)*	174 (93.0)*	237 (85.9)
Years of education	15.9 ± 3.2	15.1 ± 3.8	15.4 ± 3.4
Number of rooms in home	6.1 ± 2.6	5.8 ± 2.5	5.5 ± 2.9
Body mass index (kg/m^2)	25.9 ± 4.2**	26.8 ± 4.6	27.6 ± 5.9
Current cigarette smoker	3 (3.8)	14 (7.5)	24 (8.7)
Cumulative Illness Rating Scale score	5.4 ± 4.3	5.7 ± 3.5	5.3 ± 3.8

Values are mean ± SD or numbers (percentages). Chi-square tests and *t*-tests were used.
* $p < 0.05$ compared with controls.
** $p < 0.01$ compared with controls.

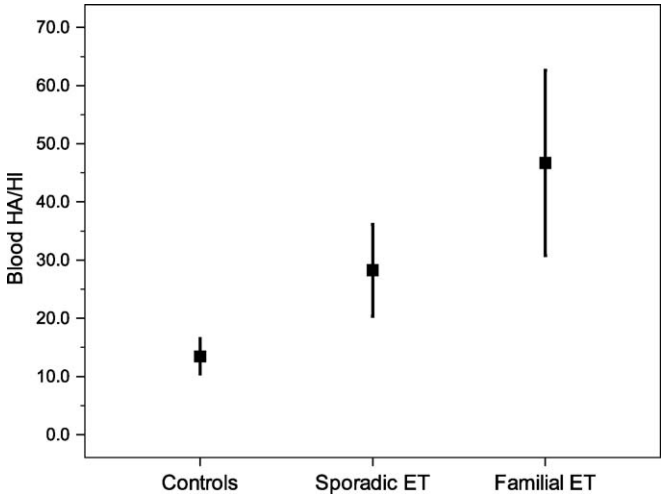

Fig. 1. Blood HA/HI ratio by diagnosis. Means (boxes) and one standard error.

(Table 2), indicating that these covariates did not account for the observed association between HA/HI ratio and diagnosis. There were 125 participants with data on the time latency between last ingestion of food or beverages and time of phlebotomy; in both the shorter time latency and longer latency strata, the HA/HI ratio remained the highest in familial ET cases (Table 2). In a sensitivity analysis, we removed potential outlying HA/HI values, excluding the top 3% of values; the results were similar. The HA/HI ratio was highest in familial ET (13.3 ± 32.2), intermediate in sporadic ET (10.1 ± 20.8), and lowest in controls (8.9 ± 23.5) (Kruskal–Wallis $p = 0.027$).

We assessed in ET cases whether use of ET medications or ethanol could have influenced their HA/HI ratio. There was no association with medication use (categorized as none, occasional, and daily, Kruskal–Wallis $p = 0.16$) or grams of ethanol consumed per day (Spearman's $r = 0.02$, $p = 0.80$). In familial ET cases, there was no association between HA/HI ratio and tremor severity (Spearman's $r = 0.08$, $p = 0.48$), age of tremor onset (Spearman's $r = -0.04$, $p = 0.73$), or tremor duration (Spearman's $r = 0.14$, $p = 0.24$).

4. Discussion

Aside from genetic factors (Higgins et al., 1997; Gulcher et al., 1997; Clark et al., 2010; Shatunov et al., 2006) non-genetic (Jiménez-Jiménez et al., 2007; Salemi et al., 1998; Louis, 2001) factors are likely to play a role in the etiology of ET (Louis et al., 2008a). Indeed, environmental factors are thought to play a significant role in several late life neurological diseases, including Alzheimer's disease and PD (Gorell et al., 1999; Racette et al., 2001; Baldereschi et al., 2008; Shcherbatykh and Carpenter, 2007). The contribution of environmental risk factors to disease etiology has been examined in detail in epidemiological studies of these other diseases (Gorell et al., 1999; Racette et al., 2001; Shcherbatykh and Carpenter, 2007; Morahan et al., 2007) and 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 one of the most commonly used experimental model for PD (Langston et al., 1984). By comparison, studies of such environmental toxins in ET lag far behind. It is often stated that approximately 50% of ET cases are non-familial (Lambert et al., 1999; Louis and Ottman, 1996). With a population prevalence for ET of 4.0% among persons aged 40 and older (Dogu et al., 2003), this suggests that approximately 2.0% of the population aged ≥40 years has a non-familial form of ET, yet the environmental correlates for this tremor are only now beginning to be explored.

β-Carboline alkaloids such as harmane are of interest in ET (Louis et al., 2002, 2008a). These toxins are structurally similar to MPTP (Langston et al., 1984; Smeyne and Jackson-Lewis, 2005). Like MPTP, β-carboline alkaloids are highly neurotoxic, and the administration of β-carboline alkaloids to a wide variety of laboratory animals produces an action tremor that resembles ET (Du et al., 1997). Indeed, β-carboline alkaloid administration is used as an animal model for ET and new pharmacotherapies are tested using exposed animals (Martin and Handforth, 2006; Martin et al., 2005; Krahl et al., 2004; Handforth and Krahl, 2001; Sinton et al., 1989). β-Carboline alkaloid-induced tremor shares clinical features and drug-response characteristics with ET (Fuentes and Longo, 1971; Sinton et al., 1989; Milner et al., 1995; Trouvin et al.,

Table 2
Blood HA/HI ratio in controls, sporadic ET and familial ET in different demographic and clinical strata.

	Blood HA/HI ratio		
	Controls	Sporadic ET	Familial ET
Age (years)			
Youngest tertile (≤64)	15.5 ± 63.9	54.1 ± 172.7	20.4 ± 77.7
Middle tertile (>64–73)	16.3 ± 53.4	18.8 ± 59.2	30.9 ± 65.9
Oldest tertile (>73)	6.6 ± 11.8	11.6 ± 23.7	77.3 ± 199.7
Gender			
Men	19.3 ± 72.5	20.5 ± 59.2	38.2 ± 90.2
Women	9.4 ± 25.0	37.2 ± 145.2	54.0 ± 173.1
Race			
White	14.1 ± 53.8	28.2 ± 111.3	49.1 ± 143.8
Non-white	9.4 ± 18.4	28.9 ± 48.3	1.9 ± 0.5[a]
Body mass index (kg/m^2)			
Lowest tertile (≤24.5)	6.1 ± 15.8	17.5 ± 55.9	40.7 ± 123.5
Middle tertile (24.6–28.1)	18.4 ± 73.6	43.0 ± 128.7	59.6 ± 185.7
Highest tertile (>28.1)	15.9 ± 45.5	25.9 ± 129.4	38.1 ± 74.9
Number of rooms in home			
Lowest tertile (≤4)	9.8 ± 37.0	21.0 ± 125.0	54.5 ± 100.6
Middle tertile (5–6)	13.1 ± 46.8	21.1 ± 53.0	33.8 ± 124.0
Highest tertile (≥7)	19.0 ± 67.4	42.6 ± 129.0	50.6 ± 171.6
Latency since oral intake (h)			
Less than median (≤1.8)	4.2 ± 5.2	8.7 ± 8.3	29.5 ± 36.3
Greater than median (>1.8)	24.4 ± 80.2	16.2 ± 44.2	213.5 ± 415.8[b]

[a] The number of subjects in this cell was very small; there were only 4 non-white participants with familial ET.
[b] The number of subjects in this cell was very small; there were only 5 participants.

1987; Cross et al., 1993; Rappaport et al., 1984). Also, several of the underlying brain changes are similar, including Purkinje cell loss (Du et al., 1997; Sinton et al., 1989; Milner et al., 1995; Louis et al., 2007; O'Hearn et al., 1993; O'Hearn and Molliver, 1993, 1997; Robertson, 1980). β-Carboline alkaloids are produced endogenously in the human body (Gearhart et al., 2000; Wakabayashi et al., 1997), but dietary sources are estimated to be far greater than endogenous sources (Pfau and Skog, 2004). β-Carboline alkaloids are primarily found in meat at ng/g concentrations, and cooking leads to increased concentrations (Gross et al., 1993; Skog, 1993; Layton et al., 1995). In addition to the high concentrations in meat, β-carboline alkaloids are also present in lower concentrations in many plants, including tobacco and coffee (Herraiz, 2004). The effects of chronic, low-level β-carboline alkaloid exposure are not known.

Using a sample of more than 500 individuals, we demonstrated that the blood harmane/harmine ratio was highest in familial ET cases, intermediate in cases of sporadic ET, and lowest in controls without ET. These findings lend support to the notion that the elevated blood harmane concentration observed in ET could be the result of a genetically-driven reduction in harmane metabolism. The higher concentration of blood harmane in familial than sporadic ET cases, which is something we noted in our previous study, further supports this notion (Louis et al., 2008a).

Although the mean harmane/harmine ratio was 23.4, the range was considerable, with values as low as 0.1 and as high as 987.5. The explanation for the high variance is not known, although could include inter-individual differences in ability to metabolize harmane, inter-individual differences in dietary intake of harmane vs. harmine, and other factors.

In univariate analyses, the harmane/harmine ratio correlated weakly with the number of rooms in the participant's home. This variable, used in prior publications (Louis et al., 2005a,b) is a socioeconomic indicator, although not as strong as household income or occupation. As a socioeconomic indicator, it could be a marker of weight and other health-related differences, underlying nutritional and dietary differences, or differences in other lifestyle factors.

This study had limitations. First, we did not assess fasting blood harmane concentrations because it was impractical to do so. Therefore, it is difficult to fully assess the extent, if any, to which our findings reflect a difference in dietary intake of harmane. However, data in a subsample of 61 of our controls indicated that there was no association between time elapsed from last food or beverage ingestion and blood harmane concentration (Spearman's $r = 0.12$, $p = 0.34$) or HA/HI ratio (Spearman's $r = 0.11$, $p = 0.41$), indicating that neither blood harmane nor the HA/HI ratio seemed to be a function of time since last food consumption. Also, in stratified analyses, the association between higher HA/HI ratio and familial ET remained robust in both shorter and longer time latency strata, further lessening the likelihood that dietary intake of harmane explained our results. Furthermore, there is no conceivable reason why familial ET cases should have diets higher in harmane or diets with a higher ratio of harmane to harmine than do their counterparts with sporadic ET. Second, we recognize that ET cases may not accurately report their family history information; nevertheless, we expect misclassification of cases to be non-differential and to bias our findings towards the null hypothesis. The study had several strengths. The first is the uniqueness of the question; there are no other studies that have examined this issue in ET or in other diseases. Second, we used a large sample size of more than 500 individuals that included both ET cases and controls. Finally, we were able to subdivide ET cases further by using family history information.

The basis for the elevated blood harmane concentration, particularly in familial ET, is not known, although these findings (highest harmane/harmine ratio in familial ET cases) lends support to the possibility that it could be the result of a genetically-driven reduction in harmane metabolism.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Statistical analyses

The statistical analyses were conducted by Dr. Louis.

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