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Research Article

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Genetic and Environmental Factors That Regulate Cytosolic Epoxide Hydrolase Activity in Normal Human Lymphocytes

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Abstract

To determine whether genetic mechanisms control large variations in cytosolic epoxide hydrolase (cEH) activity of unstimulated lymphocytes from normal human subjects, cEH activity was measured in (*a*) 6 sets of monozygotic (MZ) twins and 6 sets of dizygotic (DZ) twins; (*b*) 100 unrelated male subjects; and (*c*) 6 families.

The twin study revealed predominantly genetic control (H_I^2 = 0.95). Variability was markedly less within MZ (intrapair variance = 0.25) than DZ twins (intrapair variance = 6.33).

In 100 unrelated male subjects the extent of interindividual variation was 11-fold. Unimodal distribution of values among 99 subjects encompassed a sixfold range. One outlier with very high activity clearly stood apart.

Using the whole distribution curve we phenotyped members of six families. In the outlier's family, analysis of three generations suggested autosomal dominant transmission of high cEH activity. Analysis of the other 5 families and of 12 sets of twins, all from the large unimodal distribution, was consistent with either monogenic or polygenic control of variations within this mode.

Several temporal host factors, including fever, the menstrual cycle, a 24-h fast, and diurnal variations, were investigated. Fever and fasting elevated cEH activity. Diurnal variations produced no observable alteration. During the menstrual cycle irregular fluctuations occurred.

Introduction

Humans are exposed to a wide variety of chemicals foreign to the body, including natural products, food additives, industrial chemicals, and pollutants. Such xenobiotics can be metabolized by the cytochrome P-450 system to highly reactive, potentially toxic intermediates such as epoxides, which are considered hazardous because they can bind covalently to cellular components. Enzymatic degradation of these epoxides to less reactive intermediates is protective (1) and occurs by conjugation with glutathione via glutathione-*S*-transferase, as well as by hydration to the corresponding transdihydrodiol catalyzed via epoxide hydrolase (EH; 2).[1] Since the enzyme EH is toxicologically significant, factors causing inter- and intraindividual variations in its activity are important (3). For example, Spielberg and colleagues studied lymphocytes from rare subjects who developed idiosyncratic reactions in vivo to phenytoin, acetaminophen, or nitrofurantoin (4–6). They hypothesized a mutant form of microsomal EH as the molecular basis for these abnormal drug reactions, and furthermore demonstrated in lymphocytes of affected subjects increased susceptibility to lysis in vitro on exposure to the drug.

Ubiquitous in mammalian tissues, EHs have been classified biochemically into four main forms: cytosolic (cEH), microsomal (mEH), cholesterol, and leukotriene (7). These forms are distinguished by their substrate specificities, molecular weights, and immunological reactivities (7).

As a relatively accessible human tissue, blood has been used previously to investigate the properties of EH activity in humans (8–10). Our investigation of genetic and environmental factors responsible for interindividual variations in cEH activity in human lymphocytes extends several previous reports (8, 9) that document large but unexplained variations in the activity of this enzyme. Although in a given human subject lymphocytic and hepatic cEH activities do not closely correlate, factors that induce hepatic cEH induce lymphocyte cEH to a similar extent (9). In lymphocytes cEH activity is higher and exhibits more interindividual variability than mEH; also, with readily available substrates cEH is more reliably assayed than mEH (1). Recently cEH and mEH activities of lymphocytes were shown to be significantly correlated within a given subject (11). Thus, even though lymphocyte mEH activity was not measured in our study, our results with cEH in lymphocytes may also apply to mEH.

The present study applies to human EH for the first time several traditional methods to test a genetic hypothesis. The first method consists of analysis of a quantitative trait in monozygotic (MZ) and dizygotic (DZ) twins. If the results of the twin study indicate a strong genetic contribution to variation, the second method of pedigree analysis according to Mendel's first law is then used to determine the precise mode of this genetic transmission (12–14). Each family member is phenotyped for a particular quantitative trait by that subject's position on a distribution curve of values generated from unrelated subjects. Historically this approach, discussed in detail elsewhere (12–14), has been used frequently, leading to the discovery of several pharmacogenetic conditions, including atypical plasma cholinesterase (15), the acetylation polymorphism (16), glucose-6-phosphate dehydrogenase deficiency

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1. *Abbreviations used in this paper:* cEH, cytosolic epoxide hydrolase; DZ, dizygotic; EH, epoxide hydrolase; GST, glutathione-*S*-transferase; H^2, heritability; mEH, microsomal EH; MZ, monozygotic; TSO, trans-stilbene oxide.

(17), and polymorphic oxidations of debrisoquine (18), mephenytoin (19), antipyrine (20), and theophylline (21).

Methods

Selection of subjects. 160 normal subjects participated in this study (24 individuals from 6 families, 6 sets of MZ twins, 6 sets of DZ twins, 100 males who were unrelated, 6 menstruating females, and 6 postmenopausal females). Subjects were recruited from Hershey, PA, and vicinity by notices placed in the medical college. Subjects were all carefully screened: none had a history of serious illness or drug allergies, nor any recent illness. None consumed any medications or ethanol regularly. None smoked tobacco or were chronically exposed to any known inducing or inhibiting agent. For 3 d before participation in this study subjects abstained from all ethanol.

Materials. Commercially synthesized tritiated trans-stilbene oxide (TSO) of high radiospecificity (2.9 Ci/mmol) was purchased from Amersham Corp., Arlington Heights, IL.

Isolation of lymphocytes. Methodology for isolation of lymphocytes by Ficoll-Hypaque gradient separation has been described previously (22). Lymphocytes were then counted, diluted to the same concentration, and lysed by sonification (sonifier and cell disrupter; Branson Sonic Power Co., Danbury, CT) three times at 4°C for 10 s with a 1-min rest between pulses. This sonicate was then centrifuged at 105,000 g for 60 min to obtain a membrane-free supernatant. Both intra- and interindividual variation occurred in recovered lymphocyte populations. Nevertheless, day-to-day lymphocyte fluctuations unaccompanied by fever did not alter lymphocyte activity significantly in a given subject.

Assay. The cEH assay developed by Gill et al. (23) was applied to lymphocytes by Seidegård and DePierre (1). In accord with the latter (1), 2–3 million freshly isolated non–mitogen stimulated lymphocytes are routinely used in a total incubation vol of 100 μl. Activities were linear with the soluble fractions prepared from 1–6 million cells. Glutathione-*S*-transferase (GST) is also a cytosolic enzyme that reacts to degrade epoxides; therefore, the breakdown of TSO by GST must be measured and these counts subtracted from the cEH assay (23). Optimal cEH activity was obtained in 0.65 M sodium phosphate, pH 6.8, with 25 μM TSO, at a final radiospecificity of 20–30 mCi/mmol. After addition of the substrate in 1 μl of ethanol the reaction mixtures were incubated at 37°C for 60 min. The reaction was terminated by adding 200 μl of dodecane and then shaking vigorously before centrifugation to obtain phase separation (23).

GST activity was measured by the same procedure, except that the reaction was terminated by the addition of 200 μl of hexanol, which removes dihydrodiols but leaves the glutathione conjugates in the aqueous phase (23). 50-μl aliquots were taken from the aqueous phase after extraction with either dodecane or hexanol and scintillation counted.

Data analysis for reproducibility. A two-tailed paired t test was done to determine the reproducibility of cEH activity within an individual. This test was appropriate because it estimates both quantity and direction of change.

Genetic analysis of twin data. To estimate the relative contributions of genetic and environmental factors to interindividual variations in cEH activity, heritabilities (H^2) were calculated by several methods (12, 13, 24, 25).

H^2 is a statistical measure of the degree to which a phenotype is genetically influenced. Since no single unbiased estimate of H^2 in twins exists, it was calculated by four methods. The H^2 subscript denotes the method used. The formulas permit a range of values from 0 (negligible genetic control over interindividual variation) to 1 (virtually complete hereditary influence).

The first method is the standard estimate of H^2 (H_1^2) based on intrapair differences, $H_1^2 = V_{DZ} - V_{MZ}/V_{DZ}$, in which V_{MZ} and V_{DZ} represent variances within MZ and DZ twins, respectively. Variance was calculated as follows: $V = [\Sigma(\text{differences between twins})^2]/2N$, in which N is the number of twin pairs of the same zygosity.

A second method (H_2^2) uses controls generated by scrambling twin pairs (12, 13) and calculates H^2 as follows: $H_2^2 = V_{CDZ} - V_{MDZ}/V_{CDZ}$.

Other estimates of heritability, H_3^2 and H_4^2, are based on intraclass correlation coefficients: $H_3^2 = 2(r_{MZ} - r_{DZ})$, and $H_4^2 = r_{MZ} - r_{DZ}/1 - r_{DZ}$, where r_{MZ} and r_{DZ} are the intraclass correlation coefficients for MZ and DZ twins, respectively. Intraclass correlation coefficients were calculated using the following equation (12, 13): $r = \Sigma(X - a)(Y - a)/Ns^2$. Where X and Y are the values of each twin in a given twinship, a is the mean common to all MZ or DZ twin measurements, and s^2 is the variance about mean a. Intraclass correlation coefficients were also compared for twins, regardless of zygosity, living in the same household and in different households.

The degree of genetic influence can be measured by intra- and interpair variance. Intra- and interpair variance is calculated as follows: intrapair variance $= \Sigma(A - B)^2/2N$, and interpair variance $= 1/N[\Sigma(A + B)^2/2 - [\Sigma(A + B)]^2/2N]$. Variance is compared by dividing the larger variance by the smaller variance, the ratio being referred to as F.

The zygosity of twins was determined by HLA and blood group analysis.

Pedigree analysis. Pedigree analysis was performed on 6 families selected from the 100 unrelated male subjects. Selection of five families came from the ends and middle of the large unimodal distribution curve, whereas the sixth family was that of the outlier with the very high cEH activity. Phenotypes were determined by each family member's position on the distribution curve constructed from the 100 unrelated male subjects.

K_m and V_{max} study analysis. In three subjects, consisting of the outlier and two at either extreme of the large unimode, nine different substrate concentrations were used to measure specific cEH activity. Enzyme activities were calculated using Lineweaver-Burk plots of enzyme velocity and substrate concentration to determine K_m and V_{max}.

Immunoblot analysis. Immunochemical quantitation of cEH protein was accomplished through the use of antibodies against mouse liver cEH in the Western blot procedure. These antibodies were generously donated by Dr. Bruce D. Hammock. Protein concentrations were determined by the Bradford method (26) and resolved by SDS-PAGE according to Laemmli (27). After electrophoresis the resolved proteins were electrophoretically transferred to nitrocellulose sheets as described previously (28). The protein bands were probed with anti-cEH antibodies and then with anti-rabbit IgG conjugated with alkaline phosphate. The bands containing cEH protein were visualized as described by Blake et al. (29) and quantified by densitometry.

Results

Reproducibility: intra- and interindividual variations

Reproducibility of the cEH assay was explored by analysis of variance designed for repeated measurements in the six unrelated male subjects studied on four separate occasions ~ 1 wk apart. Intraindividual variation was observed to be small, each subject showing high reproducibility, resulting in an average SD for this sample population of 0.49 pmol product formed/min per 10^7 cells. Thus, the magnitude of intraindividual variation did not reach statistical significance ($P < 0.05$). By contrast, the extent of interindividual variation exceeded that of intraindividual variation threefold. These results are indicative of large interindividual and small intraindividual variations. They agree with those previously reported by Seidegård et al. (8) in 27 Swedish subjects.

Genetic factors

Twin studies. Twins served to compare the relative genetic and environmental contributions to interindividual variation of

cEH activity (Fig. 1). Intrapair variability was significantly less within MZ twins (0.25) than within DZ twins (6.33), suggesting either monogenic or polygenic control of large interindividual variation in cEH activity. Similar interpair variability of MZ twins (19.15) and DZ twins (19.37) indicated that critical environmental determinants did not differ between these two groups (Table I). Estimates of heritability for lymphocyte cEH activity indicate predominantly genetic control over interindividual variation. Values for H^2 were 0.95, 0.96, 0.98, and 0.70 for H_1^2, H_2^2, H_3^2, and H_4^2, respectively (Table I).

Intraclass correlation coefficients for twins living together in a single household regardless of zygosity (0.82) exceeded those for twins who lived in different households (0.51), but the difference did not attain the level of statistical significance by a three-way analysis of variance ($P > 0.05$).

Distribution curve in 100 unrelated individuals. The distribution curve (Fig. 2) and pedigree analysis (Fig. 3) support the hypothesis of genetic control over the large interindividual variations that we observed in cEH activity. A distribution curve was constructed for lymphocyte cEH activity in 100 unrelated male subjects with an average age of 25±5.3 yr. In Fig. 2 each bar represents the mean of two blood samples drawn from each subject ~ 1 wk apart. The mean lymphocyte cEH activity±SD of the 100 unrelated male subjects was 9.35±4.03 pmol product formed/min per 10^7 cells. An 11-fold difference occurred between the two subjects with highest (2.8) and lowest (30.4) cEH activity.

The distribution curve in Fig. 2 appears to be unimodal for 99 subjects, but the single outlier with high activity is clearly separate. The large unimodal curve could be generated if these six-fold interindividual variations in cEH activity were controlled either polygenically (by genes at multiple loci) or monogenically (by a single genetic locus with two alleles, A and a). According to the latter possibility, the 99 subjects with phenotypes in the low range of cEH activity could be assigned genotypes of aa. The single outlier with highest cEH activity could be assigned a genotype of either AA or Aa.

Pedigree analysis. 6 pedigrees containing 27 subjects are depicted in Fig. 3: the family of the subject with highest cEH activity in Fig. 2 as well as five other families selected from

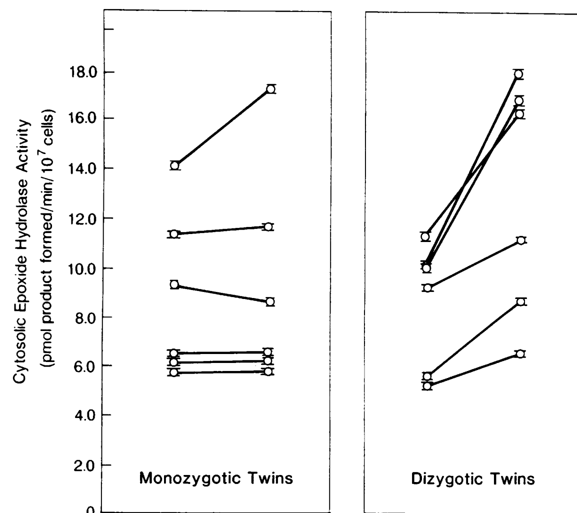

Figure 1. Lymphocyte cEH activities of six sets of male monozygotic twins and six sets of male dizygotic twins.

Table I. Heritabilities and Intra- and Interpair Variances of cEH Activity in Six Sets of MZ and Six Sets of DZ Twins

Heritability	Formula	Value
H_1^2	$\frac{V_{DZ} - V_{MZ}}{V_{DZ}}$	0.95
H_2^2	$\frac{V_{CDZ} - V_{CMZ}}{V_{CDZ}}$	0.96
H_3^2	$2(r_{MZ} - r_{DZ})$	0.98
H_4^2	$r_{MZ} - r_{DZ}$	0.70
Intrapair variance		Value
MZ = 0.25 DZ = 6.33	$\frac{\Sigma(A - B)^2}{2N}$	25.32*
Interpair variance		Value
MZ = 19.15 DZ = 19.37	$\frac{1}{N-1}\ \frac{\Sigma(A + B)^2}{2} - \frac{(\Sigma A + B)^2}{2N}$	1.01 NS

* Significantly different, $P < 0.01$.

both extremes and the middle of the large mode of cEH activity in Fig. 2. Each pedigree was consistent with a monogenic hypothesis, specifically that the very high cEH activity observed in a single pedigree (Fig. 3) is transmitted as an autosomal dominant trait. The most genetically informative pedigree (family 1) contains the subject with the highest cEH activity from the distribution curve and two consanguineous marriages of seventh and ninth cousins for marriages I and II, respectively. High cEH activity occurred in four members of family 1: I-2, II-2, III-2, and III-3. Subject III-2 was the propositus. Low cEH activity was observed in one member of family 1, as well as in all members of families 2–6. Family 1 was unusual both in its large size and in keeping extensive and careful records of births and marriages. Lymphocyte cEH activity was not determined for subject III-1 in family 1 because this subject was a young menstruating female. Since menstruating females exhibit large fluctuations in lymphocyte cEH activity, only postmenopausal females were investigated.

Environmental factors

Fever. Effects of fever on cEH activity of lymphocytes were investigated. Four male subjects, ranging in age from 23 to 35 yr, were in good general health and without known medical illnesses except for this transient febrile illness. Fever elevated lymphocyte cEH activity in each subject ($P < 0.05$; Fig. 4). The control value for subject 1 was measured both before and after the febrile episode. Nonfebrile lymphocyte cEH activities for subjects 1 and 4 represent the mean of four blood samplings. The febrile value for subject 1 shows the mean of two blood samplings; on both measurements during his illness his temperature was 39°C. Basal afebrile values for subjects 2 and 3 were determined 2 wk after recovery from illness. During the febrile state lymphocyte cEH activity was measured once for subjects 2, 3, and 4. Temperatures were measured sublingually using the same thermometer. When the mean value of the basal state of cEH activity was compared with the mean value during the febrile state, enzyme activity during fever increased 1.8-fold.

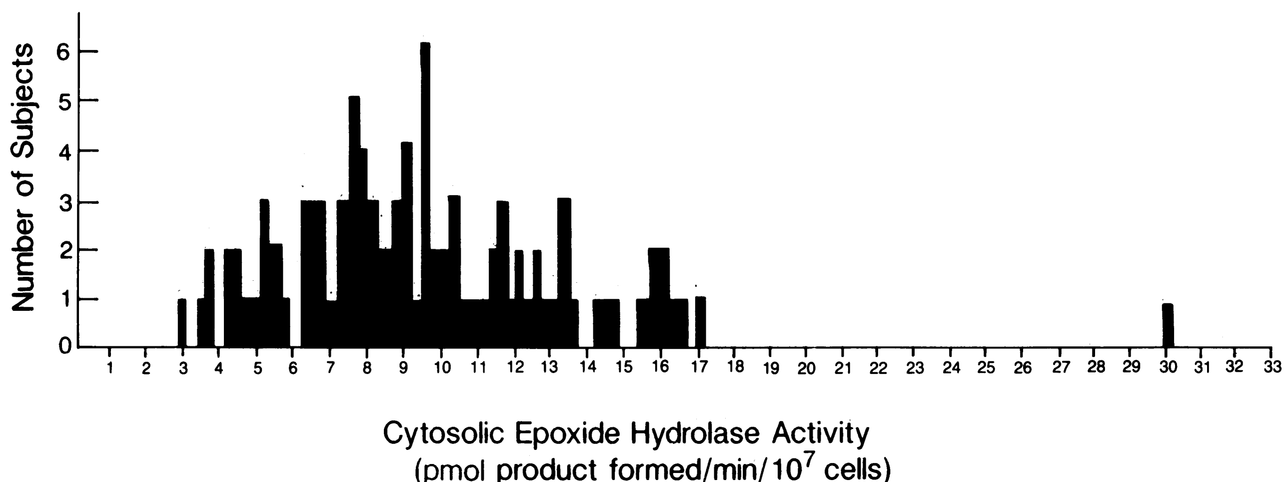

Figure 2. Frequency distribution of lymphocyte cEH activity in 100 unrelated normal male subjects. Two blood analyses were performed for each subject; the mean of these values is expressed for each subject as a bar.

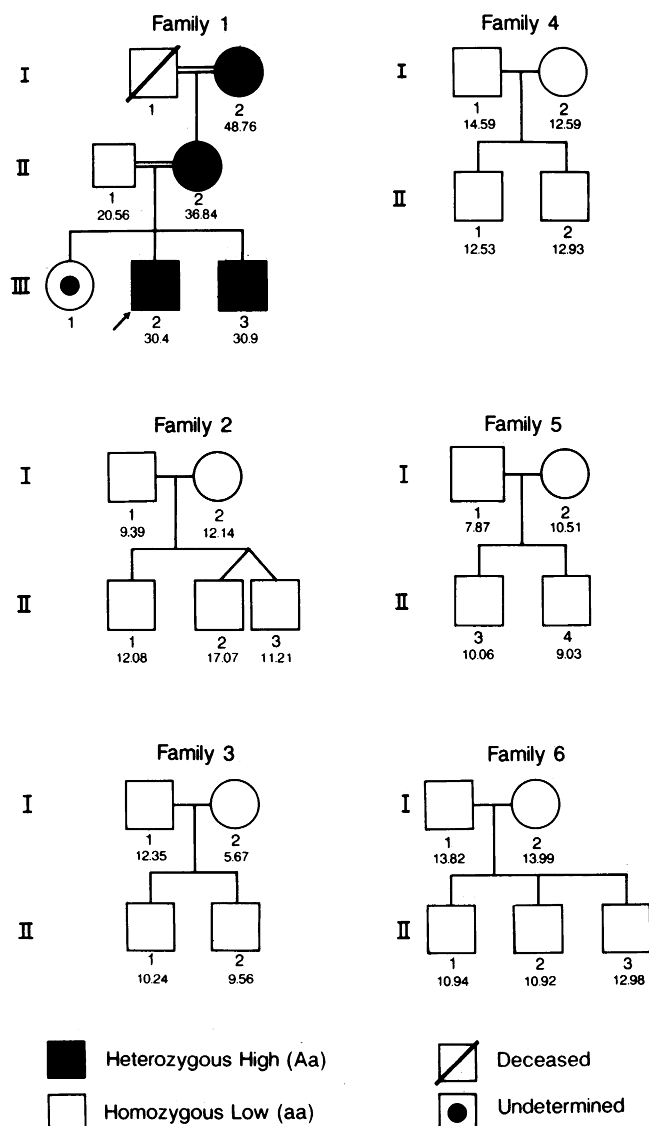

Figure 3. Pedigree analysis of six families for lymphocyte cEH activity. Each subject's cEH activity appears below the subject's symbol. The arrow indicates the propositus.

Diurnal variation. Potential diurnal variations in lymphocyte cEH activity were explored in seven normal male volunteers between the ages of 23 and 52 yr. For each volunteer on the same day blood samples were drawn and assayed at 7:00 a.m. and 7:00 p.m. No significant difference in cEH activity occurred between 7:00 a.m. and 7:00 p.m. (Fig. 5). In two subjects lymphocyte cEH activity was measured at noon and midnight. No differences occurred between these values and those obtained at 7:00 a.m. and 7:00 p.m.

Fasting. The results of a 24-h fast on lymphocyte cEH activity are depicted in Fig. 6. Subjects ate no food during this 24-h fast but took water ad lib. Control values for each subject were obtained before the 24-h fast.

A statistically significant difference ($P < 0.01$) between fasting and basal lymphocyte cEH activity was observed for the group. Fasting elevated mean lymphocyte cEH activity by 2.2-fold. The extent of this elevation ranged from 3.2-fold for subject 1 to 1.7-fold for subject 3. Subject 1 repeated the 24-h fast and a comparable 3.2-fold change occurred in the second trial.

Menstrual cycle. The pattern of lymphocyte cEH activity was measured 12 times during a single menstrual cycle of 28 d for each of six subjects, where day 0 corresponds to the first day of menses (Fig. 7). Lymphocyte cEH activities spiked and fluctuated throughout the menstrual cycle.

Postmenopausal women. Six postmenopausal females (aged 50–63 yr) were studied. In individual subjects lymphocyte cEH activity was highly reproducible (Table II) in marked contrast to menstruating females (Fig. 7). Mean values±SD of

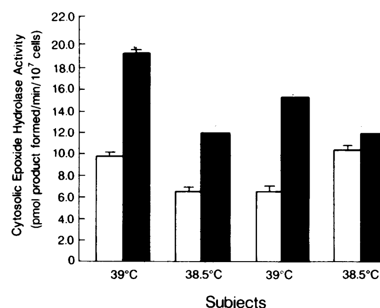

Figure 4. The effect of fever on lymphocyte cEH activity in four male subjects. □, Afebrile; ■, febrile.

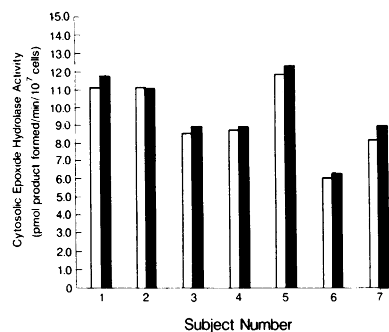

Figure 5. The effect of diurnal variation on lymphocyte cEH activity in seven male subjects. For each subject blood samples were drawn at 7:00 a.m. (□) and 7:00 p.m. (■) on the same day.

lymphocyte cEH activity in postmenopausal females (9.82±2.06 pmol product formed/min per 10^7 cells) closely resembled those of the 100 unrelated male subjects (9.35±4.03 pmol product formed/min per 10^7 cells). In menstruating females mean values±SD were 13.55±5.65 pmol product formed/min per 10^7 cells; larger fluctuations occurred than in either postmenopausal women or normal males, as indicated by the higher SD.

Molecular studies

Kinetic analysis in selected individuals. Kinetic analysis of the hydrolysis of TSO by lymphocyte cEH from three subjects, one subject having the highest activity and the other two subjects selected from both extremes of the unimodal curve, is given in the form of Lineweaver-Burk plots (Fig. 8). In these three subjects Lineweaver-Burk plots revealed that cEH activity followed Michaelis-Menten kinetics. V_{max} values were 42.73, 23.70, and 5.66 pmol product formed/min per 10^7 cells for subject 1 with high and subjects 2 and 3 with low lymphocyte cEH activities, respectively (Fig. 8). These different specific activities reflect the level of lymphocyte cEH activity measured in each subject (Fig. 2). The K_m values were 16.13, 10.53, and 3.03 μM for subject 1 with highest and subjects 2 and 3 with low lymphocyte cEH activities, respectively.

Immunochemical studies on lymphocyte cEH. Immunoblot analysis of fresh lymphocyte cEH treated with the protease inhibitor, leupeptin, migrated with purified mouse hepatic cEH; both proteins were visualized on nitrocellulose at 58 kD (Fig. 9). No differences in electrophoretic mobility or immunoreactivity appeared among subjects. Without the protease inhibitor, lymphocyte cEH degraded to a single peptide visualized on nitrocellulose at ~ 18 kD (Fig. 10). Males and females showed similar immunoreactivity of this 18-kD cEH

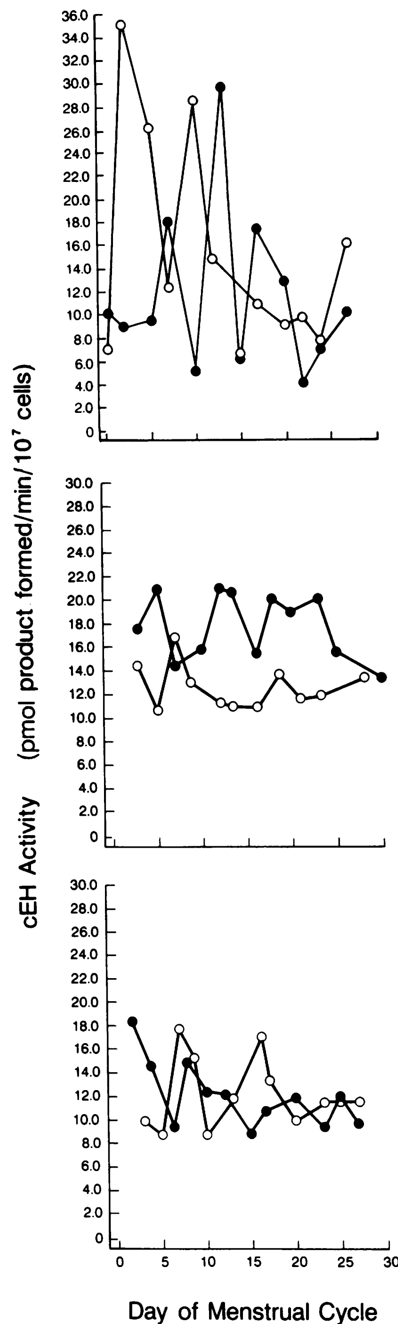

Figure 7. The effect of the menstrual cycle on lymphocyte cEH activity in six female subjects.

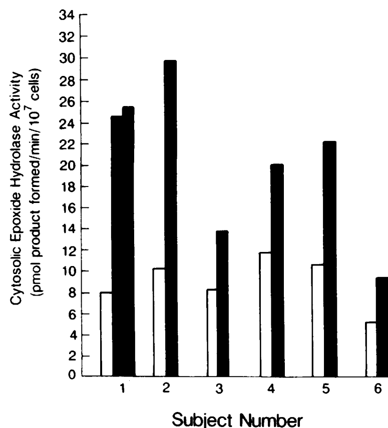

Figure 6. The effect of a 24-h fast on lymphocyte cEH activity in six male subjects. Control values were obtained before fasting; fasting samples were drawn at 8:00 a.m. One wk after the first fast, subject 1 fasted again. □, Control; ■, 24-h fast.

Table II. cEH Activity in Six Postmenopausal Women, Each Measured on Two Separate Trials

Subject	Trial No. 1	Trial No. 2	Mean	SD
	pmol product formed/min per 10^7 cells			
1	11.13	11.27	11.20	±0.10
2	7.38	7.06	7.22	±0.23
3	9.15	9.55	9.35	±0.28
4	7.61	7.90	7.75	±0.20
5	10.92	11.08	11.00	±0.11
6	12.14	12.69	12.41	±0.39

Mean±SD, 9.82±2.06; average deviation on repetition, ±0.22.

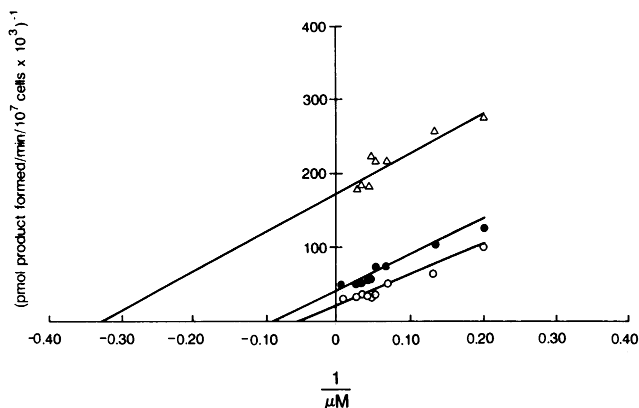

Figure 8. K_m and V_{max} for three subjects for the hydration of TSO as displayed in a Lineweaver-Burk plot. K_m values were 16.13, 10.53, and 3.03 μM, and V_{max} values were 42.73, 23.70, and 5.66 pmol product formed/min per 10^7 cells for subjects 1 (○), 2 (●), and 3 (△), respectively.

protein with the exception of the single outlier, subject III-2 (Fig. 2), whose 18-kD peptide showed twofold more immunoreactivity than other subjects as measured by reflective densitometry.

Discussion

A two-tiered method to reveal pharmacogenetic polymorphisms (13) was applied to elucidate causes of large interindividual variations in cEH activities of human lymphocytes: a twin study as an initial screen to test for the operation of genetic factors followed by a family study to test a monogenic hypothesis. Our results in normal male twins carefully selected to be under uniform environmental conditions suggest that large interindividual variations in lymphocyte cEH activity arise predominantly from genetic factors (Table I). These could be either monogenic or polygenic. The second part of the two-tiered system, a pedigree analysis, was performed on the family of the single outlier, as well as on 5 other families selected from the 99 subjects in the large unimodal distribution. Family members were phenotyped according to their position on a distribution curve generated from unrelated normal male subjects. For the outlier and his family a monogenic hypothesis was indicated. Analysis of the other five families and the twins, all drawn from the large unimodal curve, is consistent with either monogenic or polygenic control of the sixfold cEH variations on this portion of the curve (Fig. 2).

A fundamental assumption in the application of the twin method to estimate and compare the relative contributions of genetic and environmental factors to interindividual variations in lymphocyte cEH activity is that the environments of MZ and DZ twins are nearly uniform with respect to critical factors that influence cEH activity (30). The magnitude of genetic influence on a continuously distributed variable is indicated by intra- and intertwin variances. Markedly smaller intrapair variance in MZ (0.25) compared with DZ (6.33) twins ($P < 0.01$) indicates that genetic factors play a significant role in maintaining large interindividual differences in lymphocyte cEH activity. Although twins living apart had lower intratwin correlation (0.51) than those living together (0.82), this difference did not reach statistical significance ($P > 0.05$). Accordingly, a firm role for environmental contributions to phenotypic variation could not be drawn from this twin study.

Our twin study suggests that certain genetic factors control large phenotypic variations in lymphocyte cEH activity, variations that exist despite use of subjects under carefully controlled uniform environmental conditions. Pedigree analysis was then performed to identify a specific Mendelian mode of transmission of these genetic factors.

One family was identified with four members exhibiting lymphocyte cEH activities three times higher than the mean value (9.35 pmol product formed/min per 10^7 cells) of the 100 subjects in Fig. 2. The propositus was the subject with the highest cEH activity (30.40) in Fig. 2. Analysis of his family revealed similarly elevated lymphocyte cEH activity in a male sibling of the propositus (31.90), as well as his mother (36.84) and grandmother (48.76) (Fig. 3). All family members were healthy and had not been exposed to any previously recognized inducing agent or other environmental factor that might be responsible for this enhanced activity. In this pedigree, transmission of very high cEH activity for three generations, as well as the distribution curve shown in Fig. 2, is consistent with autosomal dominant expression of the trait. The question arises as to whether the phenotype of high cEH activity is neutral or confers a benefit or disadvantage. It has been suggested that under some environmental circumstances studied in vitro low cEH activity, as determined by the Ames test, can prove harmful (2).

Another issue is whether in this family the genetic factor implicated is limited to cEH or also affects other genes, such as those that regulate cytochrome P-450 isozymes. Antipyrine clearance measured in the propositus (III-2 in Fig. 3) after a single oral antipyrine dose of 18 mg/kg was in the normal

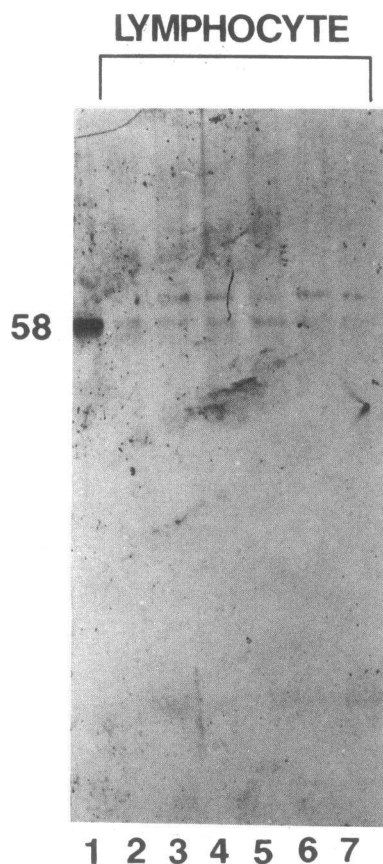

Figure 9. Immunoblot of purified mouse hepatic cEH protein and leupeptin-treated human lymphocyte cEH protein. The 58-kD band corresponds to the migration of purified mouse hepatic cEH protein (lane *1*) and to lymphocyte cEH from three male subjects. The male subject with highest lymphocyte cEH activity (lanes *6* and *7*) exhibited immunoreactivity similar to that of the two subjects (lanes *2* and *3* for one subject, and lanes *4* and *5* for the other subject) selected from the extremes of the large mode in Fig. 2.

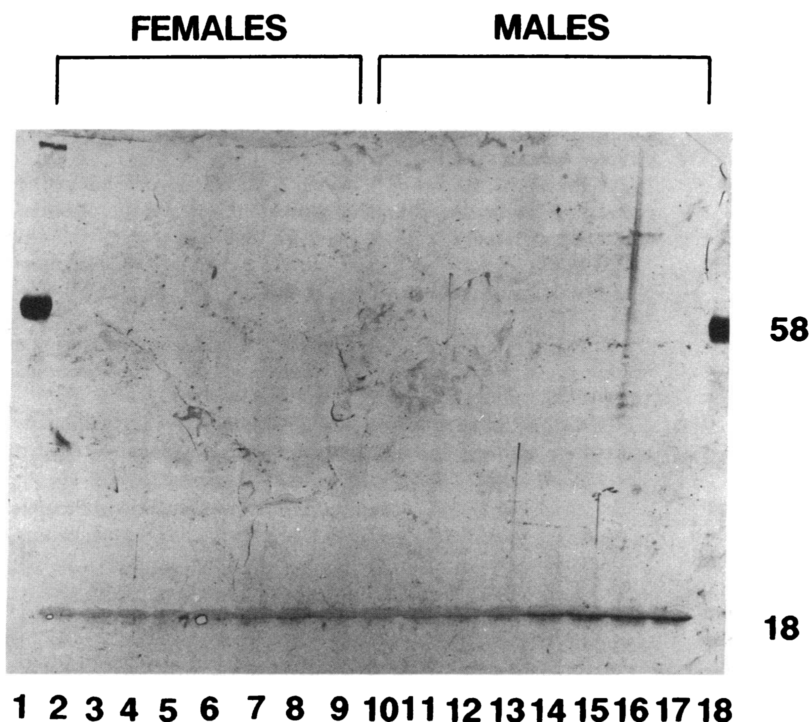

Figure 10. Immunoblot of purified mouse hepatic and human lymphocyte cEH protein in four subjects (two females and two males). The 58-kD band corresponds to the migration of purified mouse hepatic cEH (lanes *1* and *18*). Protease inhibitors were not added to lymphocyte preparations. Note enhanced immunoreactivity of the 18-kD protein in the subject with the highest lymphocyte cEH activity (lanes *14–17*).

range, suggesting that three of his hepatic cytochrome P-450 isozymes were functioning normally (20, 30–32). Thus, his elevated lymphocyte cEH activity appears to be an isolated alteration, neither correlated with, nor genetically linked to, function of several hepatic cytochrome P-450 isozymes.

The broad unimodal distribution curve shown in Fig. 2 can be interpreted in several ways. For example, a broad unimodal distribution can arise from polygenic or monogenic control of phenotypic variation. Further analysis of this large mode is required to establish its precise genetic makeup, as well as the role of environmental factors. If the trait for high cEH activity in the outlier and his family is transmitted as an autosomal dominant, then all individuals in the broad unimodal range shown in Fig. 2 could possess a single genotype, aa, and the extensive range of values within that single mode could arise from environmental differences that cause diverse phenotypic expressions of the same genotype. As an alternative to the monogenic hypothesis, the large unimodal curve could be generated by polygenic mechanisms, which are recognized to be extremely sensitive to environmental perturbation (13, 14). Accordingly, we attempted to identify environmental factors that might alter a subject's basal lymphocyte cEH activity.

Fever and fasting were shown to elevate a given subject's lymphocyte cEH activity, whereas diurnal variation appeared to produce no obvious alteration (Figs. 4–6). The mechanisms by which fever and fasting enhance lymphocyte cEH activity are unclear, and future studies should address their effects on the transmission pattern in the outlier's family, on Lineweaver-Burk plots, and on cEH immunodensity. Such studies might clarify whether the defect in the outlier's family arises from abnormal regulation of gene expression analogous in certain respects to fasting and fever. After our studies were completed, increases in mitochondrial and cEH activity were described in livers from mice starved for 24 and 48 h (33). Large irregular variations in lymphocyte cEH activity during the menstrual cycle were first observed by Seidegård (personal communication) and attributed to hormonal variations. Perhaps changes in other hormones associated with fever and fasting may explain the variations in cEH activity we observed in these states. We confirmed and extended Siedegård's observation (Fig. 7), finding that lymphocyte cEH activity spiked and fluctuated during the menstrual cycle. In contrast, males and postmenopausal women (Table II) are highly reproducible. Moreover, mean values of lymphocyte cEH activity for postmenopausal females shown in Table II are similar to those for the males.

Kinetic analysis of three male subjects selected from different positions in the distribution curve (Fig. 2) yielded V_{max} values as expected for subjects with different lymphocyte cEH activities. Our values for both K_m and V_{max} are of similar magnitude to those reported by Seidegård (8). K_m values determined by standardizing enzyme activity between subjects are needed, as well as kinetic studies on purified lymphocyte cEH.

Densitometric analysis of immunoblots of lymphocyte cEH protein in four subjects revealed approximately equal amounts of immunoreactivity in three subjects. The subject with highest cEH activity exhibited twofold more cEH immunoreactivity than the other three (Fig. 10). Since this difference was not observed in immunoblots from cells exposed to leupeptin (Fig. 9), high cEH activity could arise either from increased proteolytic processing of cEH to a hypothesized more active 18-kD form or from decreased degradation of this form.

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