Genetic Polymorphism for Human Platelet Thermostable Phenol Sulfotransferase (TS PST) Activity

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ABSTRACT

Platelet TS PST basal activity and thermal stability were measured in blood samples from 237 individuals in 50 nuclear families. Significant correlations were found among first degree relatives, confirming the previously reported familial aggregation of TS PST basal activity and thermal stability. Commingling analysis of basal TS PST activity provided evidence for multiple component distributions, and after transformation to remove skewness, segregation analysis supported a major gene hypothesis. For TS PST thermal stability, commingling analysis also provided evidence for multiple component distributions. However, segregation analyses were equivocal with regard to the presence of a major gene for thermal stability, since support for a major gene model depended on skewness. Bivariate commingling analysis, which examined thermal stability by simultaneously considering basal activity and activity after heating, suggested that genotypes, as defined by the inferred component distributions for TS PST activity, differ in thermal stability. A three-allele model is proposed as one hypothesis that may account for the combined results of basal activity and thermal stability. The results of this study indicate that a major gene polymorphism in conjunction with polygenic inheritance plays an important role in the regulation of both level of activity and thermal stability of this important drug-metabolizing enzyme in humans.

SULFATE conjugation is an important metabolic pathway for many drugs. Phenol sulfotransferase (PST, EC 2.8.2.1) catalyzes the sulfate conjugation of a large number of phenolic and catechol drugs, xenobiotic compounds and neurotransmitters (DODGSON 1977; ROY 1977; WEINSHILBOUM 1986b). The discovery that PST activity is present in an easily accessible human tissue, the blood platelet (HART *et al.* 1979), served as one important stimulus for the study of this enzyme in man. Human platelets contain two independently regulated forms of PST that differ in their physical properties, substrate specificities and sensitivity to inhibitors (REIN, GLOVER and SANDLER 1981, 1982; REITER and WEINSHILBOUM 1982a; REITER *et al.* 1983). One form of the enzyme is thermostable and preferentially catalyzes the sulfate conjugation of "simple" phenols such as *p*-nitrophenol and phenol. This form of the enzyme has been referred to as either "TS" (thermostable) or "P" (phenol metabolizing) PST (REIN, GLOVER and SANDLER 1981; REITER and WEINSHILBOUM 1982a; REITER *et al.* 1983). The other form is thermolabile and preferentially catalyzes the sulfate conjugation of dopamine and other monoamines. It has been referred to as the "TL" (thermolabile) or "M" (monoamine metabolizing) form of PST (REIN, GLOVER and SANDLER 1981; REITER and WEINSHILBOUM 1982a). Platelet and hepatic TL and TS PST can be separated by ion exchange chromatography (REITER *et al.* 1983; CAMPBELL, VAN LOON and WEINSHILBOUM 1987).

PST has been studied in the human platelet primarily because of the possibility that its biochemical properties and regulation in that accessible tissue might reflect those of the enzyme in organs more directly involved in drug and neurotransmitter metabolism (WEINSHILBOUM 1986a,b). The biochemical properties of platelet PST are very similar to or identical with those of the enzyme in human brain, liver and small intestine (YOUNG *et al.* 1984; CAMPBELL and WEINSHILBOUM 1984; SUNDARAM and WEINSHILBOUM 1985; CAMPBELL, VAN LOON and WEINSHILBOUM 1987). In addition, individual variations in platelet TS PST activity are significantly correlated with individual differences in TS PST activity in other human tissues, including cerebral cortex, liver and small intestinal mucosa (YOUNG *et al.* 1985; CAMPBELL and WEINSHILBOUM 1986; SUNDARAM, TUCKER and WEINSHILBOUM 1986).

Since thermal stability is a sensitive measure of variation in protein structure (LANGRIDGE 1968; WEINSHILBOUM 1981), that property of TS PST has

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also been studied in platelet preparations and in other tissues. In these studies thermal stability was measured by comparing enzyme activity in a heated sample to that in an unheated sample, a so-called heated/control (H/C) ratio (VAN LOON and WEINSHILBOUM 1984). Significant individual variations in platelet TS PST thermal stability were reported (VAN LOON and WEINSHILBOUM 1984)— variations that reflect individual differences in TS PST thermal stability in other tissues such as cerebral cortex and intestinal mucosa (YOUNG *et al.* 1985; SUNDARAM, TUCKER and WEINSHILBOUM 1986). In the platelet, samples with low thermal stability also have lower basal levels of enzyme activity (VAN LOON and WEINSHILBOUM 1984). These observations demonstrate that individual variations in the basal activity of TS PST and individual variations in the thermal stability of the enzyme measured in the platelet reflect variations in both basal TS PST activity and thermal stability in other, less accessible tissues.

Preliminary studies performed with a small number of monozygotic and dizygotic twins showed high heritability of variation in both TS and TL PST activities in the platelet (REVELEY *et al.* 1982/1983). We recently reported high heritability for TL PST in the platelet based on a study of families (PRICE *et al.* 1988) and raised the possibility of a major gene polymorphism for platelet TL PST. A significant familial aggregation of platelet TS PST thermal stability has also been reported (VAN LOON and WEINSHILBOUM 1984). In the present study we examined platelet TS PST basal activity and thermal stability in blood samples from nuclear families to clarify the mechanism or mechanisms by which genes influence TS PST activity in humans.

We begin by describing summary statistics for basal activity and H/C ratios for TS PST. Next we describe commingling and segregation analyses, first for basal activity, then for H/C ratio as a measure of thermal stability. Finally, we describe a bivariate commingling analysis that considers basal and heated activities simultaneously.

MATERIALS AND METHODS

Subjects: Blood samples were obtained from 237 individuals who were members of 50 nuclear families identified without respect to enzyme activity (KEITH *et al.* 1983; VAN LOON and WEINSHILBOUM 1984). These studies were approved by the Mayo Clinic Institutional Review Board, and written informed consent was obtained from all participants.

The distribution of sibship size in the final sample used in the segregation analysis is given in Table 1. Sibships ranged in size from 1 to 7 with the majority having 2 or 3 sibs. The sibs ranged in age from 4 to 20 yr with a mean age of 13.4 yr. Parents ranged in age from 32 to 53 yr and had a mean age of 40.2 yr. Data for basal TS PST activity were unavailable for two mothers.

Platelet isolation: Blood samples were obtained in 7-ml Vacutainer tubes that contained 10.5 mg disodium EDTA. Platelets were isolated, counted, and prepared as described in detail previously (VAN LOON and WEINSHILBOUM 1984). Platelet homogenates were stored at −20°. TS PST activity and thermal stability are unchanged under these conditions of storage.

TABLE 1

Sibship size of families included in segregation analysis of platelet TS PST

Sibship size	No. of families	No. of individuals
1	3	8[a]
2	20	80
3	19	94[a]
4	4	24
5	2	14
6	1	8
7	1	9
Totals	50	237

[a] Missing information on the mother in one family.

TS PST assay: TS PST activity was measured by the method of FOLDES and MEEK (1973) as modified by ANDERSON and WEINSHILBOUM (1980) and by REITER *et al.* (1983). The assay is based on the sulfation of *p*-nitrophenol, a model substrate for TS PST, by the enzyme in the presence of [^{35}S]-3′-phosphoadenosine-5′-phosphosulfate (PAPS), the sulfate donor for the reaction. Details of the assay procedure have been described elsewhere (VAN LOON and WEINSHILBOUM 1984). One unit of PST activity represented the formation of 1 nmol of product per hour of incubation. The results were expressed per 10^8 platelets, since that method has been shown to result in less variation than expression of the activity per milligram platelet protein (ANDERSON *et al.* 1981).

Thermal stability: TS PST thermal stability was measured as described by REITER and WEINSHILBOUM (1982a). Specifically, frozen platelet homogenates were thawed, diluted and preincubated for 15 min in a shaker water bath at 44°, while control aliquots were kept at 4°. All samples were placed on ice immediately after the preincubation step. TS PST activity was then measured in both control and "heated" samples. Thermal stability was expressed as a heated to control (H/C) ratio.

Family resemblance: Standard interclass correlations were computed for mothers and fathers and for parents and offspring. For parent-offspring correlations, all possible pairings were included. Intraclass correlations were computed for siblings using standard methods, *i.e.*, a random effects ANOVA allowing for unequal sibship size. Expected mean squares were computed as in SNEDECOR and COCHRAN (1980).

Commingling analysis: The presence of a major gene polymorphism affecting a quantitative trait will result in skewness or multimodality in the population phenotypic distribution. A method of assessing multimodality described by MACLEAN *et al.* (1976) uses maximum likelihood to fit mixtures of two or three normal distributions to data, and compares the fit obtained with that using a single distribution. The following parameters are estimated by the computer program SKUMIX (MACLEAN *et al.* 1976): the mean (u) of the distribution; the variance (V), assumed to be the same for each component distribution; the proportion of admixture (q), which corresponds to gene frequency in a major gene model; the displacement (t, in standard deviation

TABLE 2

Sample characteristics and distribution statistics for platelet TS PST activity and H/C ratio for 237 members of 50 families

Variable		N	Mean	Standard deviation	Skewness	Kurtosis
TS PST	Basal Activity	237	0.46	0.29	1.70	4.42
TS PST	Standardized by age, generation, sex	237	0.00	1.00	1.69	4.41
TS PST	Power Transformed ($b = -3.01$)	237	−0.27	0.84	0.15	−0.83
H/C	Heated/Control ratio	237	0.47	0.13	−0.92	0.71
H/C	Standardized by age, generation, sex	237	0.00	1.00	−0.89	0.70
H/C	Power Transformed ($b = -1.08$)	237	−0.16	0.95	0.02	0.26
Age		237	24.51	13.60	0.37	−1.60

units of the combined distribution) between the means of the upper and lower distributions; and the relative displacement (d) of the intermediate distribution. The value of d determines whether there are two ($d = 0$ or 1) or three component distributions ($0 < d < 1$). When only the mean and variance are estimated ($d = t = q = 0$) there is only one distribution. The relative sizes of the component distributions were assumed to be Hardy-Weinberg proportions, *i.e.*, $(1 - q)^2$, $2q(1 - q)$, and q^2. An additional parameter (b) is needed when transformation of the data is required to remove or reduce skewness in the component distributions. We used the Box and Cox (1964) transformation, $y = (r/b)[(x/r + 1)^b - 1]$, with $r = 6$ so that for every observation x, $(x/r + 1)$ was positive. In the limit as $b \to 0$, $y \to r \ln(x/r + 1)$.

To deal with the relationship between basal and heated activities, we also conducted a bivariate analysis following the approach taken by Spielman and Weinshilboum (1981) for catechol-*o*-methyl transferase (COMT). We used a bivariate version of SKUMIX (Price and Stunkard 1989) which is parameterized as in the univariate version, and which analogously allows for up to three joint distributions ($j = 1,3$) of the bivariate observations (x_i, $i = 1,2$), with corresponding means, variances and correlations. The means of up to three component distributions were modeled as a function of two sets of parameters (t_i, d_i and u_i, $i = 1,2$), one set for each of the marginal distributions. The variances of the component distributions can be assumed to be equal or estimated separately (V_{ij}, $i = 1,2$, $j = 1,3$). As in the original version, q is the measure of distribution admixture. The correlation coefficients (ρ_j, $j = 1,3$) between x_i scores, *i.e.*, heated and control TS PST values, in the component distributions can be set to be equal, can be estimated separately for each joint distribution, or can be set to zero. With three distributions, up to 16 parameters may be estimated. We distinguish this version of SKUMIX by calling it BIVAR. Hypothesis testing was carried out by comparing alternative models using a likelihood ratio test.

Segregation analysis: Complex segregation analysis was used to assess the evidence for major gene and polygenic components in the transmission of TS PST basal activities and H/C ratios. This method tests components of a genetic model that includes a two allele autosomal major gene, polygenic inheritance and random environmental factors. We used a version of the computer program POINTER that incorporates three transmission probabilities into the mixed model (Lalouel and Morton 1981; Lalouel *et al.* 1983).

The mixed model parameters are: the overall mean (u); the total variance (V); the variance attributable to polygenic inheritance (h^2); the gene frequency (q) for the high activity allele (a); the displacement (t) between means of homozygotes at the major locus measured in standard deviation units; the relative displacement (d) of the heterozygote mean ranging from 0.0 (recessive) to 1.0 (dominant); and probabilities of transmitting the low activity allele (A) for the three genotypes ($t_{AA;A}$, $t_{Aa;A}$, $t_{aa;A}$), which are assumed to be 1.0, 0.5 and 0.0, respectively, under the Mendelian hypothesis. $t_{Aa;A}$, for example, is the probability that a heterozygote parent (Aa) transmits the allele (A) for low activity.

A likelihood ratio test was used to compare competing models. Evidence for a major locus component in transmission of the trait was assessed by comparing the likelihood for a model that includes both major locus and polygenic components, the full model, with that for the polygenic model without the major locus, *i.e.*, determining whether the hypothesis of "no major locus component to transmission" can be rejected. Evidence for a polygenic component in transmission was assessed by comparing the likelihood for the full model with the major locus model without polygenic inheritance, *i.e.*, determining whether the hypothesis of "no polygenic component to transmission" can be rejected. It is well known that other within family influences, if they exist, will be absorbed by the estimate of polygenic heritability.

Segregation analysis was applied to nuclear families that were ascertained without regard to enzyme activity. Thus, there were no probands and no pointers. The computer programs POINTER, SKUMIX and BIVAR all use the non-linear optimization routine GEMINI (Lalouel 1979). Joint likelihoods of parents and children were used in POINTER.

RESULTS

Sample characteristics: The basal TS PST activity distribution for all relatives was positively skewed and positively kurtotic. The distribution of H/C ratios was negatively skewed and positively kurtotic. Descriptive statistics are given in Table 2. Neither basal TS PST activities nor H/C ratios were significantly correlated with age, sex or generation. The frequency distributions of basal TS PST activity and H/C ratio, standardized and adjusted for age, sex, and generation are shown in Figures 1 and 2 (bar graphs). The variables were adjusted by regression, *i.e.*, by computing standardized residuals, using the covariates directly or as dummy variables. Because of the sensitivity of segregation analyses to these kinds of covariates, we routinely adjust variables prior to segregation analysis,

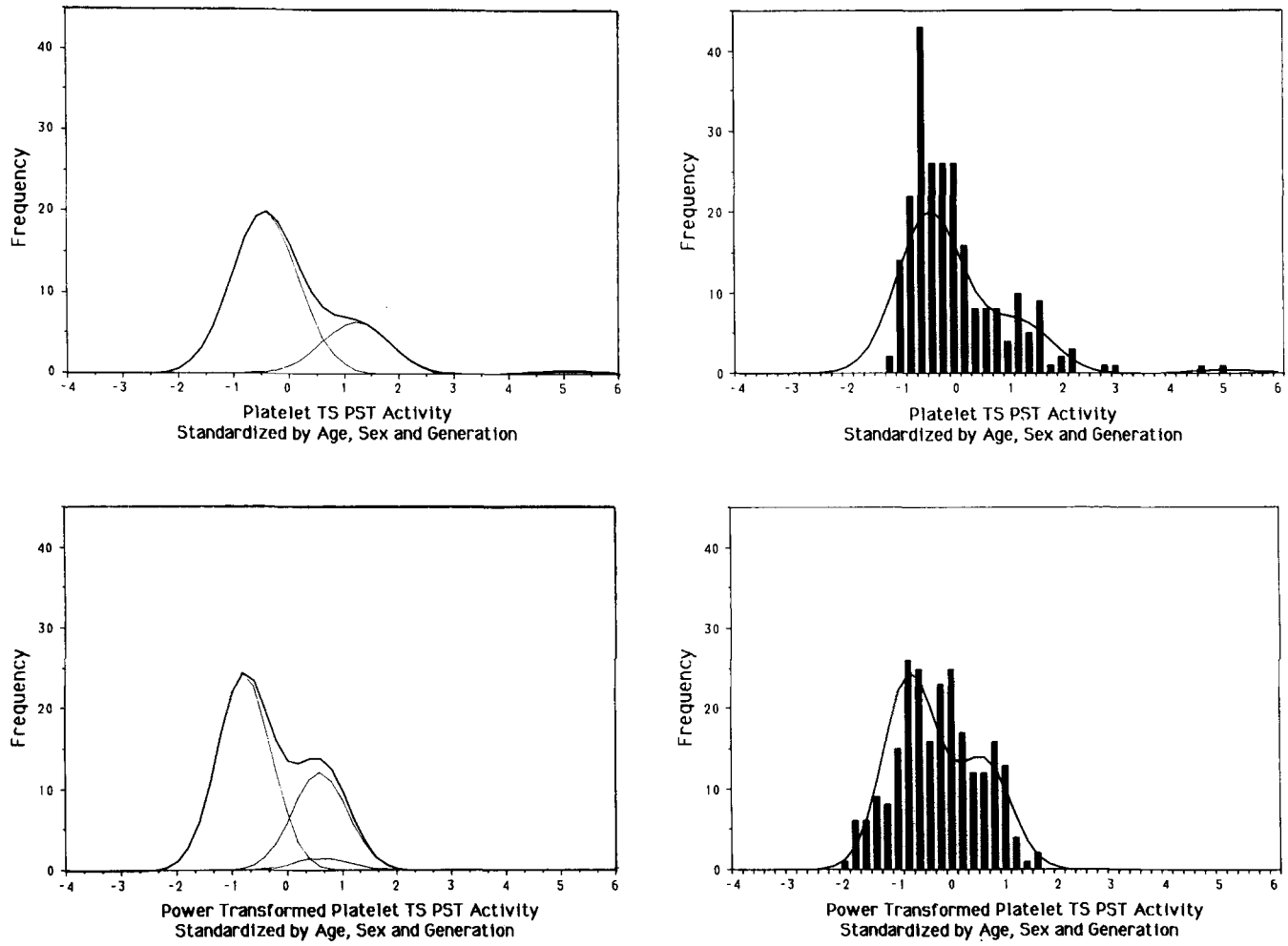

FIGURE 1.—Observed frequency distribution (bar graphs) of untransformed (upper portion) and power transformed, $b = -3.01$ (lower portion) TS PST activity. Expected component distributions (thin lines) for each TS PST activity genotype, and the total distribution (bold line), which is the sum of the three-component genotypic distributions with age, sex and generation effects removed. Expected genotypic means and distribution parameters are based upon major locus parameters from the Mendelian mixed model which were: [$u = 0.05$, $V = 1.36$, $d = 0.30$. $t = 5.53$, $q = 0.14$, $g(3) = -0.45$, $g(2) = 1.21$, $g(1) = 5.08$] for the untransformed data and [$u = -0.29$, $V = 0.68$, $d = 0.98$, $t = 1.40$, $q = 0.20$, $g(3) = -0.78$, $g(2) = 0.59$, $g(1) = 0.62$] for the power transformed ($b = -3.01$) data.

even if the effects are so small as not to be statistically significant.

Family resemblance: Family correlations for basal platelet TS PST activities and H/C ratios are presented in Table 3. Correlations between parents were low, and only one was significant ($r = -0.29$, $P < 0.05$). Parent-offspring interclass correlations were 0.33 for basal activity and 0.41 for thermal stability. Intraclass correlations among siblings were 0.55 for basal activity and 0.54 for thermal stability. Power transformation to remove skewness used values of b drawn from the commingling analysis presented below. Transformation had little effect on the magnitudes of the correlations.

There was an apparent sex effect on correlations for H/C ratio both with and without transformation to remove skewness. Sisters were significantly more highly correlated than were brothers, and mother-daughter correlations were higher than father-daughter correlations (Z test of independent correlations) For basal TS PST activity without transformation, sisters were more highly correlated than were brothers, and for this reason the combined sibling correlations exceeded parent-offspring values. The pattern of correlations does not correspond in any obvious way to one expected from sex-linked transmission or sex-influenced expression. Thus, we feel that it is appropriate to examine models of autosomal major gene inheritance of TS PST.

TS PST Basal Activity

Univariate commingling analysis: For the untransformed TS PST data there was evidence for three distributions ($\chi^2_{(1)} = 58.44$, $P < 0.001$, Table 4). The power transformation significantly improved the fit of one distribution ($\chi^2_{(1)} = 103.10$, $p<0.001$). However, for transformed TS PST two distributions ($\chi^2_{(2)} = 23.92$, $P < 0.001$) but not three distributions were supported. Thus, the commingling analysis indicated

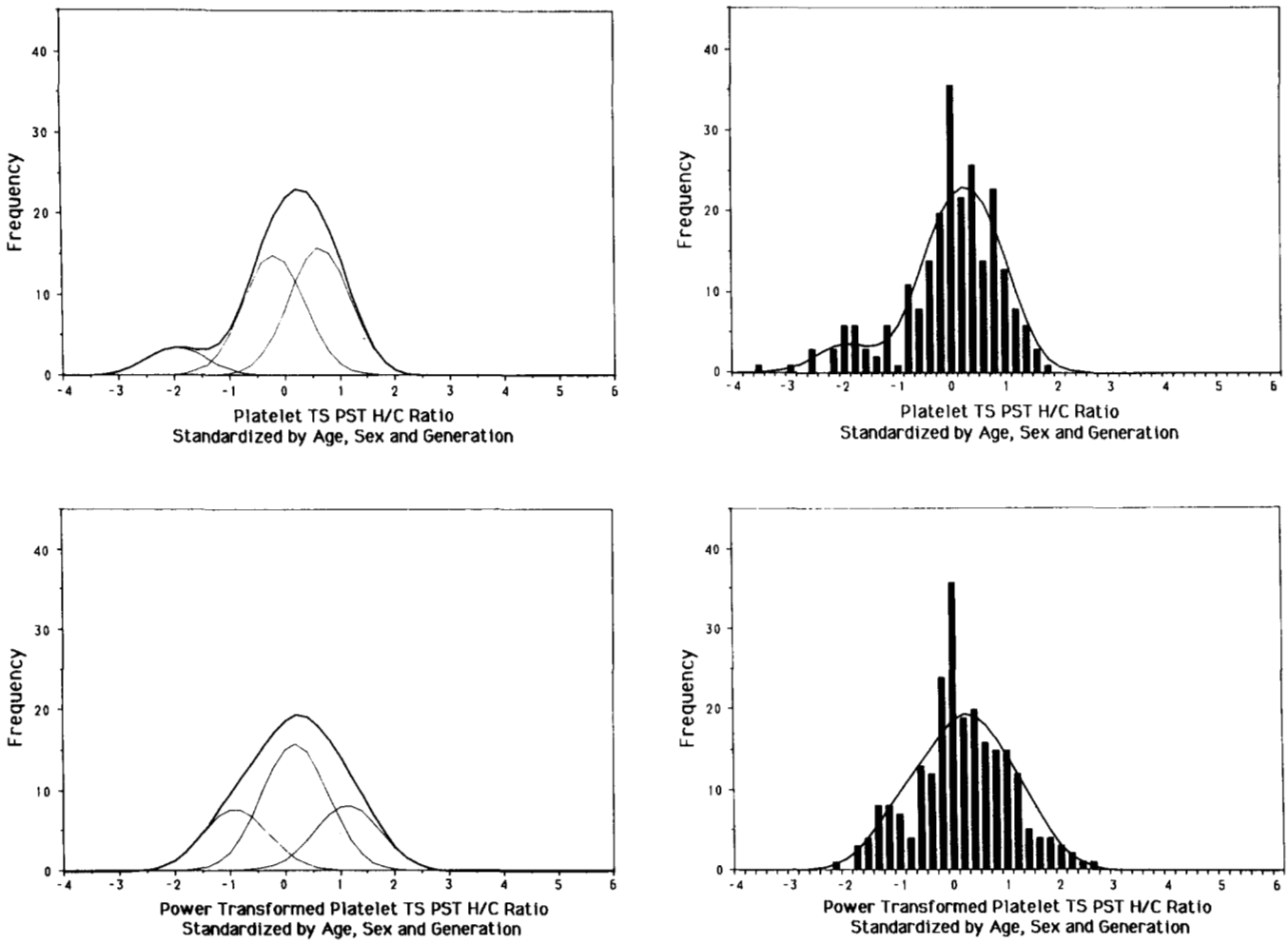

FIGURE 2.—Observed frequency distributions (bar graphs) of untransformed (upper portion) and power transformed, $b = -1.08$ (lower portion) H/C ratio. Expected component distributions (thin lines) for each H/C ratio genotype, and the total distribution (bold line), which is the sum of the three-component genotypic distributions with age, sex and generation effects removed. Expected genotypic means and distribution parameters are based upon major locus parameters from the Mendelian mixed model were: [$u = 0.01$, $V = 0.91$, $d = 0.68$, $t = 2.61$, $q = 0.68$, $g(3) = -1.97$, $g(2) = -0.18$, $g(1) = 0.64$] for the untransformed data and [$u = 0.17$, $V = 0.88$, $d = 0.53$, $t = 2.04$, $q = 0.51$, $g(3) = -0.90$, $g(2) = 0.19$, $g(1) = 1.14$] for the power transformed ($b = -1.08$) data.

that evidence for a third distribution depended upon skewness in the untransformed TS PST data.

Segregation analysis: Table 4 summarizes the results of the segregation analysis performed with the TS PST data after power transformation ($b = -3.01$, which removed skewness when one distribution was assumed). The likelihood was significantly decreased by removing the polygenic component ($\chi^2_{(1)} = 48.12$, $P < 0.001$) and by removing the major locus component ($\chi^2_{(3)} = 55.70$, $P < 0.001$) from the mixed model. The Mendelian mixed model did not differ significantly from one in which the transmission probabilities were estimated $\chi^2_{(3)} = 2.22$). When the transmission parameters were constrained to be equal it was possible to reject this model of no transmission ($\chi^2_{(2)} = 54.24$, $P<0.001$). Thus, commonly accepted criteria (LALOUEL *et al.* 1983) for acceptance of major gene transmission of TS PST were met. A major gene model with a gene frequency of 0.20 and polygenic heritability of 0.34 was supported. The lower left panel of Figure 1 illustrates the theoretical distribution for the Mendelian mixed model.

When skewness is present in the distribution of a

TABLE 3

Family correlations for platelet TS PST activity and H/C ratios

				Power transformed	
	N	TS PST	H/C	TS PST	H/C
Mother-father	48	−0.2391	−0.1056	−0.2912	−0.0591
Siblings	139	0.5475	0.5372	0.5054	0.4960
Brothers	70	0.2048	0.2888	0.4316	0.1361
Sisters	69	0.7615	0.6067	0.5617	0.6211
Parent-offspring	274	0.3329	0.4126	0.3595	0.4199
Parent-daughter	136	0.4099	0.3783	0.3857	0.3927
Parent-son	138	0.2481	0.4648	0.3257	0.4576
Mother-daughter	67	0.4188	0.5775	0.5004	0.6143
Mother-son	68	0.2543	0.4920	0.3708	0.5458
Father-daughter	69	0.4042	0.1718	0.2903	0.1443
Father-son	70	0.2426	0.4698	0.2872	0.4031

All values adjusted for age, generation, and sex.

quantitative trait, it is most appropriate to transform data to a normal distribution to guard against false inference of a major gene (LALOUEL *et al.* 1983). We also present an analysis of untransformed data, because its scale allows for easier biochemical interpretation.

The results of the segregation analysis of the untransformed data for basal TS PST activity are also summarized in Table 4. The likelihood was significantly decreased by removing the polygenic component ($\chi^2_{(1)} = 36.40$, $P < 0.001$) and by removing the major gene component ($\chi^2_{(3)} = 106.78$, $P < 0.001$) from the mixed model. However, the transmission parameters differed significantly from their Mendelian values ($\chi^2_{(3)} = 29.08$, $P < 0.001$). A model of no transmission of major genotype was rejected ($\chi^2_{(2)} = 49.89$, $P < 0.001$). The best fitting Mendelian model for the untransformed data is presented for comparison in Figure 1. The theoretical genotypic distributions for the Mendelian mixed model are illustrated in the upper left portion of the figure. The model includes a major gene with a frequency of 0.14 for the high activity allele and heritable ($h^2 = 0.25$) polygenic background variation.

H/C Ratio

Univariate commingling analysis: The values of the H/C ratio were reverse scored (multiplied by -1) after standardization ($\mu = 0$, $\sigma = 1$) and prior to the commingling analyses so that both the TS PST and H/C variables were positively skewed. Only the power transformation parameter (b) reflects this reversed scale. The reported commingling and segregation analysis parameters reflect the direction of the original scale.

For the untransformed H/C data there was evidence for two distributions ($\chi^2_{(2)} = 39.10$, $P < 0.001$, Table 4) The power transformation significantly improved the fit of one distribution ($\chi^2_{(1)} = 35.20$, $P < 0.001$). For transformed H/C ratio three distributions were supported ($\chi^2_{(3)} = 8.74$, $P < 0.05$ (three over one distribution), $\chi^2_{(1)} = 3.90$, $P < 0.05$ (three over two distributions). Thus, the commingling analysis supported three distributions for the H/C ratio data.

Segregation analysis: Table 4 summarizes the results of the segregation analysis performed with the H/C ratio data after power transformation ($b = -1.08$, which removes skewness when one distribution is assumed). The likelihood was significantly decreased by removing the polygenic component ($\chi^2_{(1)} = 13.79$, $P < 0.001$) but was not decreased by removing the major gene component ($\chi^2_{(3)} = 5.23$). The Mendelian mixed model did not differ significantly from one in which the transmission probabilities were estimated ($\chi^2_{(3)} = 3.71$). Thus, a model with high polygenic heritability (0.84) accounted for the transformed H/C data adequately. The theoretical distribution for the Mendelian mixed model is presented in the lower left panel of Figure 2 for comparative purposes.

The results of the segregation analysis of the untransformed H/C ratio data are also summarized in Table 4. The likelihood was significantly decreased by removing the polygenic component ($\chi^2_{(1)} = 13.24$, $P < 0.001$) and by removing the major gene component ($\chi^2_{(3)} = 44.12$, $P < 0.01$) from the Mendelian mixed model. The transmission parameters did not differ significantly from their Mendelian values ($\chi^2_{(3)} = 1.82$, $P = 0.62$) and the model of no Mendelian transmission was rejected ($\chi^2_{(2)} = 26.80$, $P < 0.001$). The best fitting Mendelian model for the untransformed data was one which included a major gene with a frequency of 0.68 for the allele for high thermal stability and heritable ($h^2 = 0.20$) polygenic background variation. The upper left panel of Figure 2 illustrates the theoretical genotypic distributions for the Mendelian mixed model.

Bivariate commingling analysis: Previous studies have shown that platelet samples with low thermal stabilities also have lower average TS PST basal activities (VAN LOON and WEINSHILBOUM 1984). That same relationship was also found in the present study (Figure 3). The ratio of heated to control activity (H/C) has already been shown to be a stable individual characteristic over time (WEINSHILBOUM 1986a,b). A bivariate analysis was undertaken to determine whether a joint analysis would yield a clearer picture of the genetic relationship between TS PST basal activity and activity after heating.

The results of the bivariate analyses are summarized in Table 4. When one joint distribution was assumed, the maximum likelihood estimate of the correlation coefficient was high ($\rho = 0.97$, $\chi^2_{(1)} = 655.76$, $P < 0.001$). Two distributions fit better than one, and three distributions fit better than two. Distribution parameters differed only slightly for heated and control values, but the effect on the likelihoods was significant. Correlations in the component distributions differed only when the variances were assumed to be equal. There was little information for estimating the variances and correlation coefficients for the high activity homozygote distribution. When all variances and both sets of marginal distribution parameters were estimated, the likelihood was appreciably increased, but the surface was extremely flat. With all 16 parameters it was not possible to obtain a convergent solution with these data.

When marginal distribution parameters and component distribution variances were assumed to be equal (an 8 parameter model) good convergence was achieved. The correlations between heated and control values in the high (0.81) and intermediate (0.77)

TABLE 4

Summary of genetic analyses of platelet TS PST activity and H/C ratio

	Basal TS PST activity						H/C ratio					
	Untransformed			Transformed			Untransformed			Transformed		
Commingling analysis												
N of distributions		3			2			2			3	
Sizes of component distributions	0.78	0.21	0.01	0.66	0.34		0.60	0.40		0.12	0.46	0.42
Segregation analysis												
Polygenic inheritance		Yes			Yes			Yes			Yes	
h^2		0.81			0.34			0.20			0.84	
Major gene supported		No*			Yes			Yes			No*	
Sizes of component distributions	0.74	0.24	0.02	0.64	0.32	0.04	0.10	0.44	0.46	0.24	0.50	0.26
Bivariate analysis												
N of distributions		3										
Sizes of component distributions	0.78	0.21	0.01									
Correlations coefficients	0.91	0.79	0.81									

[a] Component distribution sizes, based on major genotype frequencies, included for comparative purposes.

distributions differed significantly from the correlation in the low activity distribution (0.91) ($\chi^2_{(2)} = 9.98$, $P < 0.01$)

DISCUSSION

The results of our study confirm and extend a previous report (REVELEY *et al.* 1982/1983), based on a small number of twins, that there is a genetic component to variation in the regulation of human platelet TS PST activity. In addition, our results indicated the presence of a major gene polymorphism for TS PST basal activity. The commingling and segregation analyses supported the presence of a major gene effect on basal platelet TS PST activity. Segregation analysis of transformed TS PST activity values under a mixed model indicated that the data were best explained by a common, dominantly expressed ($d = 0.98$) major gene with a frequency of 0.20 for the high activity allele. There was heritable polygenic background variation ($h^2 = 0.34$) It should be noted that the apparent similarity of this gene frequency with that reported earlier for TL PST (PRICE *et al.* 1988) is coincidental. Previous reports found no correlation between TL and TS PST activities in the same individual (REITER and WEINSHILBOUM 1982a; ANDERSON and JACKSON 1984).

The results of our present analyses also extend earlier reports of a significant familial aggregation of platelet TS PST thermal stability (VAN LOON and WEINSHILBOUM 1984) by demonstrating a genetic component to variation to this enzyme characteristic. However, our results for thermal stability were equivocal with respect to support for a major gene. There was evidence for multiple component distributions based on analyses of H/C ratios, both with and without transformation. Furthermore, segregation analyses of the untransformed data suggested a major gene polymorphism. For the power transformed H/C ratios, a major locus model was not supported. In that case, the most parsimonious model was that of polygenic inheritance with a high heritability ($h^2 = 0.77$).

The possibility that there may be "thermostable" and "thermolabile" subtypes of the TS form of PST has been suggested on the basis of studies of both human platelet and other tissues including cerebral cortex, small intestine and liver (VAN LOON and WEINSHILBOUM 1984; YOUNG *et al.* 1985; SUNDARAM, TUCKER AND WEINSHILBOUM 1986; CAMPBELL, VAN LOON AND WEINSHILBOUM 1987). In the present study, we examined the relationship of heated and control activity in the TS form of PST. The correlations between these measures of activity were lower for the moderate and high activity component distributions, presumably heterozygotes and homozygotes, respectively, for a high activity allele. This finding suggested that there may be distinct genotypes that differ in response to thermal treatment. In summary, the bivariate commingling analysis suggested that allelic enzyme forms, as defined by TS PST *activity* might differ in thermal *stability*. However, the segregation analyses were equivocal as to whether thermal stability, as measured by H/C ratio, is influenced by a major gene, since support for a major gene effect depended on skewness.

Transformation to a normal distribution prior to segregation analysis is the customary procedure to protect against false inference of a major gene

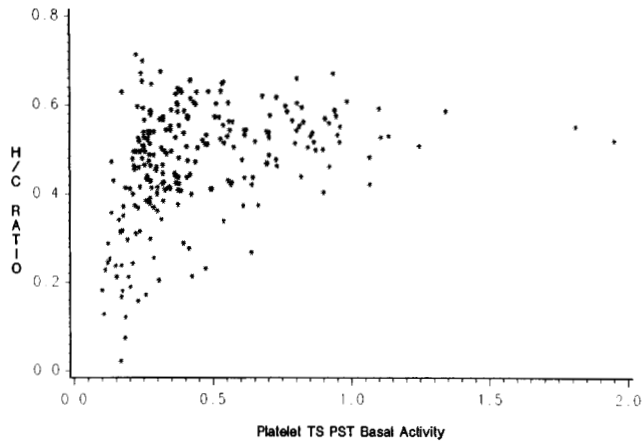

FIGURE 3.—Scatter plot of platelet TS PST basal activity (control) and H/C ratios.

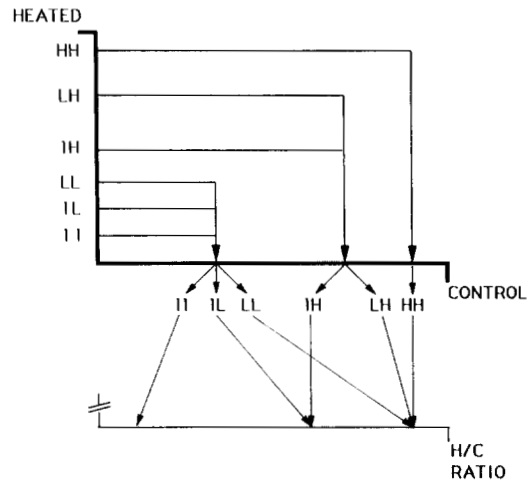

FIGURE 4.—Hypothesized relationship of genotype to heated, control and H/C ratio phenotypes for platelet TS PST activity. A structural gene with three allelic forms is hypothesized: *H*, a high activity thermostable allele; *L*, a low activity thermostabile allele; and *l*, a low activity thermolabile allele. Relative magnitudes for the three quantitative phenotypes are indicated for each of the six possible genotypes.

(MACLEAN *et al.* 1976; GO, ELSTON AND KAPLAN 1978; EAVES 1983). However, transformation also results in considerable loss of power to detect a major gene effect when one is present. DEMENAIS, LATHROP and LALOUEL (1986) examined nuclear family data simulated either by a multifactorial model with skewness or by a major gene model. Testing all three transmission probabilities for departures from the expected Mendelian values and assuming equal transmission probabilities greatly reduced the number of false inferences of a major gene due to skewness. Transformation, on the other hand, resulted in a loss of more than 50% of the power to detect a major gene.

In the analyses of H/C ratio, it is possible that an additive major gene could have mimicked polygenic inheritance. Simulation studies have indicated that there is limited power to differentiate an additive major gene effect (as suggested here for H/C ratios) from polygenic inheritance (MACLEAN *et al.* 1976). Thus, it is possible that the evidence from the untransformed data correctly indicated a major gene effect on the H/C ratio.

Our analyses provided evidence for a two-allele system for basal TS PST activity. They also provided evidence (although more equivocal) for a two-allele system for H/C ratio. However, the genotype frequencies, corresponding to component distribution sizes, differed markedly (Table 4) Thus, a single two-allele system could not account for genetic variation in both TS PST basal activity and H/C ratio.

We considered two hypotheses to account for these results. Under the first hypothesis there would be two separate loci, *e.g.*, one a structural gene for TS PST with alleles whose products differ in thermal stability and another a modifier/regulatory gene that influences basal activity of the structural gene product. Under this hypothesis it would be necessary, in view of the results shown in Figure 3, to assume that only certain structural gene variants are sensitive to the effects of the modifier/regulatory gene. Thus, if two loci were involved, an epistatic interaction would also have to be postulated.

An alternative hypothesis would explain the observed results with a single locus, avoiding the need for a special interaction. Under this hypothesis there would be one allele, *H*, for high basal activity, with a thermostable enzyme product. For low basal activity, there would be two alleles, *L* and *l*, with products that are indistinguishable under basal conditions. However, the product of allele *L* would be thermostable (like that of *H*), while the product of *l* would be thermolabile. Figure 4 is a diagram of the relative magnitudes of the quantitative phenotypes of basal (control) activity, heated activity, and H/C ratio for the six possible genotypes.

A key feature of this model is that the genotypes would contribute differently to component distributions for basal TS PST activity and H/C ratio. Thus, this model could explain the apparent difference in frequencies for the TS PST basal activity and thermal stability "genes" (Table 4). Another important feature of the model is that alleles for high basal TS PST activity (*H*) would be homogeneous with respect to thermal stability. However, alleles for low basal TS PST activity (*L*, *l*) would differ in thermal stability and therefore yield highly variable H/C ratios, as was observed (Figure 3). This model also accounts for the apparent differences in thermal stability of the high, intermediate and low activity component distributions found in the bivariate commingling analysis.

In summary, our results indicate that a major gene polymorphism, in conjunction with polygenic inheritance, is involved in the control of TS PST. The extent to which the same or different genes determine

basal activity and thermal stability of the enzyme cannot be resolved by a quantitative analysis of existing data and will require the application of biochemical and molecular methods. Biochemical data already exist which support a genetic polymorphism for TS PST thermal stability in the human liver (MAIDAK, CAMPBELL and WEINSHILBOUM 1986; CAMPBELL, VAN LOON and WEINSHILBOUM 1987; WEINSHILBOUM 1988).

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