Genetic Segregation Analysis of Red Blood Cell (RBC) Histamine N-Methyltransferase (HNMT) Activity

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Methylation is an important pathway in the biotransformation of many drugs, neurotransmitters, and xenobiotic compounds. Histamine N-methyltransferase (HNMT) catalyzes the Nτ-methylation of histamine and structurally related compounds. Measurement of HNMT activity in the RBC makes it possible to access variation in the enzyme activity that may reflect differences in less accessible tissues such as brain. Previously reported high family correlations for RBC HNMT activity suggested that genetic inheritance plays a major role in the regulation of variation in this enzyme. In the present study we completed complex segregation analyses of RBC HNMT activity of 241 individuals in 51 nuclear families that were randomly ascertained through children in the Rochester, Minnesota public school system in order to characterize the mode of inheritance of this important enzyme. We found evidence for major gene influence on the regulation of RBC HNMT activity. Both transformed and untransformed data support the presence of Mendelian major gene segregation, but the gene frequency differences do not indicate a direct correspondence between genotypes inferred from the two sets of analyses. Analyses of the skewed untransformed data indicated the presence of a relatively rare (Q = 0.121) additive major gene for high activity, with the three overlapping genotype distributions representing 77, 21, and 2% of individuals. Analyses of the normalized transformed data indicated the presence of a common (Q = 0.71) additive major gene for high activity, with the three overlapping genotype distributions accounting for 9, 41, and 50% of individuals. The analyses of transformed data give the best fit as well as the most parsimonious Mendelian major gene model. However, we cannot rule out the possibility of multiple alleles, and analyses of untransformed data provide some support for a third allele. Molecular studies will be needed to validate and characterize the alleles that regulate RBC HNMT activity levels in humans. © 1993 Wiley-Liss, Inc.

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INTRODUCTION

Histamine N-methyltransferase (HNMT, EC 2.1.1.8) catalyzes the N\textsubscript{\texttau}-methylation of histamine and structurally related compounds [Schayer, 1959; Brown et al., 1959]. Histamine is a neurotransmitter in the mammalian central nervous system [Hough and Green, 1984], and the major pathway for the metabolism of histamine in the brain is N\textsubscript{\texttau}-methylation catalyzed by HNMT [Schwartz et al., 1971; Schayer and Reilly, 1973]. HNMT activity is also present in an easily accessible human cell, the red blood cell (RBC) [Axelrod and Cohen, 1971; Van Loon et al., 1985]. Many other methyltransferase enzymes are present in the human RBC, levels of activity of several of these enzymes are controlled by inheritance, and measurement of their relative levels of activity in the RBC has made it possible to predict individual differences in the activities of these same enzymes in less accessible tissues [Weinshilboum, 1984; 1989].

We previously reported high family correlations for RBC HNMT activity (mid-parent with oldest son and oldest daughter, 0.49 and 0.51, respectively) and suggested that genetic inheritance might play an important role in the regulation of individual variation in levels of activity of this enzyme [Scott et al., 1988]. Because of the importance of histamine as a neurotransmitter and as a mediator of gastric acid secretion and allergic response, it is important to understand the basis for individual differences in its metabolism. In the present study, we performed complex segregation analyses of levels of RBC HNMT activity in order to characterize the possible mode of inheritance of this important enzyme activity in an easily accessible human cell, the RBC. The present studies represent an additional step in the determination of the possible role of inheritance in individual differences in the metabolism of histamine in humans.

SUBJECTS AND METHODS

Subjects

The subjects in this study consisted of 241 individuals in 51 families that were randomly ascertained through children in the Rochester, Minnesota public school system. The sample has been described previously [Scott et al., 1988]. The families consisted of 69 sons with a mean age of 14.4 years, 75 daughters with a mean age of 14.0 years, 46 fathers with a mean age of 47.6, and 51 mothers with a mean age of 40.0. The most common family size was two or three children.

Blood Samples

Blood was collected in 5-ml heparinized Vacutainer tubes (Becton-Dickson, Rutherford, NJ). RBCs were “washed” and lysed as previously described. Samples of lysate were stored at −80°C for 6–12 months. Storage under these conditions has no effect on RBC HNMT activity [Van Loon et al., 1985].

RBC HNMT Activity Assay

RBC HNMT activity was measured by the method of Van Loon et al., [1985]. The assay was based on the conversion of histamine to N\textsubscript{\texttau}-methylhistamine with (\textsuperscript{14}C-methyl)-S-adenosyl-L-methione as the methyl donor. Histamine was present in the reaction mixture at a final concentration of 300 μM. Blank samples contained no histo-
mine. The radioactive product of the enzyme reaction was extracted into chloroform, the chloroform was evaporated under a stream of air, 10 ml of 3A70 liquid scintillation counting fluid (Research Products International Corp., Mount Prospect, IL) was added, and radioactivity was measured in a Beckman LS-7500 liquid scintillation counter (Beckman Instruments, Palo Alto, CA). The RBC HNMT activity assay procedure has been described in detail elsewhere [Van Loon et al., 1985]. One unit of enzyme activity represented the formation of 1 pmol of Nα-methylhistamine per hour of incubation at 37°C. Each assay was preformed in triplicate, and values reported are averages of those three determinations. A pooled sample of RBC lysate was assayed with each set of samples as a standard, and RBC HNMT activity in the pooled lysate was used to correct for between assay variation.

**Specific Data Analyzed**

Statistics describing the HNMT activity data are described in Table 1. RBC HNMT values are somewhat lower and less variable in the children than in their parents, and all distributions are positively skewed. Before analyses were performed, we removed effects of gender, generation, and age on RBC HNMT activity by computing multiple linear regression residuals. All residuals were standardized within gender and generation to a mean of 0.0 and a standard deviation of 1.0

**Genetic Analysis**

**Commingling analysis.** Model parameters were estimated by maximum likelihood using a variation of the computer program SKUMIX. The full model is described in detail elsewhere [MacLean et al., 1976; Price and Stunkard, 1989]. Here we only used the program to find the best power transformation of HNMT activity to remove skewness prior to computing family correlations and conducting segregation analysis. We used the Box and Cox [1964] transformation as implemented in SKUMIX, $y = (r/b)((x/r + 1)^b - 1)$, with $r = 6$ so that for every observation $x$, $(x/r + 1)$ was positive. In the limit as $b \rightarrow 0$, $y \rightarrow r \ln(x/r + 1)$. The value of $b$ that minimized skewness in a single distribution was selected. The value of $b$ was $-0.251$.

**Segregation analysis.** Complex segregation analysis was used to assess the evidence for major gene and polygenic components in the transmission of HNMT activity. 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].

<table>
<thead>
<tr>
<th>TABLE 1. Characterization of RBC HNMT Activity (Units/ml RBC) and Age (Years) of Nuclear Family Members by Gender and Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>$N$</td>
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<tr>
<td>Mean HNMT activity</td>
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<tr>
<td>SD</td>
</tr>
<tr>
<td>Skewness</td>
</tr>
<tr>
<td>Kurtosis</td>
</tr>
<tr>
<td>Mean age</td>
</tr>
</tbody>
</table>
The mixed model parameters were the overall mean \((U)\), the total variance \((V)\), the proportion of variance attributable to polygenic inheritance \((H)\), the gene frequency \((Q)\) for the high HNMT 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 HNMT activity allele \((A)\) for the three genotypes \((tAA;A, tAa;A, tAa;A)\), which are assumed to be 1.0, 0.5, 0.0, respectively, under the Mendelian hypothesis. The parameter \(t_{Aa;A}\) for example, is the probability that a heterozygote parent \((Aa)\) transmits the allele \((A)\) for low HNMT activity.

Likelihood comparisons were made to test 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 mixed 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 mixed model with the major locus model without polygenic inheritance, i.e., determining whether the hypothesis of "no polygenic component to transmission" can be rejected.

Segregation analysis was applied to nuclear families that were randomly ascertained. Thus, there were no probands and no pointers. The computer programs POINTER and SKUMIX both use the nonlinear optimization routine GEMINI [Lalouel, 1979]. Joint likelihoods of parents and children were used in POINTER.

Hypothesis tests were accomplished by comparing likelihoods of the observed data, given two paired models. Twice the difference in log-likelihoods between models is distributed approximately as a chi square with degrees of freedom equal to the difference between models in the numbers of free parameters [Morton et al., 1983]. Since some tests of support involved constraints at boundaries rather than at interior regions of the parameter space, the assumed chi square distribution may hold only approximately.

RESULTS

Segregation Analysis

**Untransformed data.** The results from fitting various models to the untransformed data are summarized in Table II. There was strong evidence for genetic transmission of RBC HNMT activity, since the model of no transmission \((U,V)\) was rejected when compared with models allowing for familial transmission. The genetic transmission models were supported over the model of no transmission \((U,V)\) as follows: The polygenic model \((U,V,H, \chi^2_{(1)} = 26.72, P<10^{-6})\); and the Mendelian major gene model \((U,V,D, Q,T, \chi^2_{(3)} = 42.50, P < 10^{-8})\). The Mendelian mixed model \((U,V,D,Q,T,H)\) improved the fit over both the polygenic model \((U,V,H, \chi^2_{(3)} = 33.48, P<10^{-6})\) and the Mendelian major gene model \((U,V,D,Q,T, \chi^2_{(1)} = 18.15, P<10^{-4})\). Thus, the model requires both polygenic and major gene components. The Mendelian mixed model \((V,U,D,Q,T,H)\) did not differ from an additive model \((U,V,D = 0.5, Q,T,H \chi^2_{(1)} = 1.64, \text{nonsignificant})\). Furthermore, the heterozygote transmission probability did not differ from Mendelian expectation (0.50) for either the full Mixed model or for the additive mixed model \((\chi^2_{(1)} = 3.50 \text{ and } 3.20, \text{ respectively, both nonsignificant})\). The heterozygote transmission probability differed from its expected Mendelian value
TABLE II. Best Fitting Segregation Models for RBC HNMT Activity

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters</th>
<th>$V$</th>
<th>$U$</th>
<th>$D$</th>
<th>$T$</th>
<th>$Q$</th>
<th>$H$</th>
<th>$\theta_A \theta_A$</th>
<th>$-2 \ln (L)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untransformed data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V,U$</td>
<td>1.00</td>
<td>+0.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>682.93</td>
</tr>
<tr>
<td>$V,U,H$</td>
<td>1.11</td>
<td>+0.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.88</td>
<td>—</td>
<td>—</td>
<td>656.21</td>
</tr>
<tr>
<td>$V,U,D,T,Q$</td>
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<td>+0.01</td>
<td>0.48</td>
<td>2.48</td>
<td>0.28</td>
<td>0.00</td>
<td>0.50</td>
<td>0.00</td>
<td>640.88</td>
</tr>
<tr>
<td>$V,U,T,Q$</td>
<td>1.02</td>
<td>+0.01</td>
<td>0.50</td>
<td>2.41</td>
<td>0.28</td>
<td>0.00</td>
<td>0.50</td>
<td>0.00</td>
<td>640.97</td>
</tr>
<tr>
<td>$V,U,D,T,Q,H$</td>
<td>1.34</td>
<td>—0.08</td>
<td>0.30</td>
<td>4.00</td>
<td>0.12</td>
<td>0.67</td>
<td>0.50</td>
<td>0.00</td>
<td>622.73</td>
</tr>
<tr>
<td>$V,U,D,T,Q,H,\theta_A \theta_A$</td>
<td>1.26</td>
<td>—0.08</td>
<td>0.52</td>
<td>2.72</td>
<td>0.11</td>
<td>0.67</td>
<td>0.50</td>
<td>1.00</td>
<td>619.23</td>
</tr>
<tr>
<td>$V,U,T,Q,H,\theta_A \theta_A$</td>
<td>1.22</td>
<td>—0.09</td>
<td>0.50</td>
<td>2.90</td>
<td>0.10</td>
<td>0.67</td>
<td>0.88</td>
<td>0.00</td>
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<td>—0.08</td>
<td>0.50</td>
<td>2.73</td>
<td>0.12</td>
<td>0.67</td>
<td>0.50</td>
<td>0.00</td>
<td>624.37*</td>
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<tr>
<td>Transformed data*</td>
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<td></td>
<td></td>
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<tr>
<td>$V,U$</td>
<td>1.02</td>
<td>—0.10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>688.00</td>
</tr>
<tr>
<td>$V,U,H$</td>
<td>1.13</td>
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<td>0.86</td>
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<td>2.14</td>
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<td>0.00</td>
<td>0.50</td>
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<td>645.50</td>
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<tr>
<td>$V,U,D,T,Q,\theta_A \theta_A$</td>
<td>1.01</td>
<td>—0.12</td>
<td>0.45</td>
<td>2.15</td>
<td>0.70</td>
<td>0.00</td>
<td>0.45</td>
<td>0.00</td>
<td>645.28</td>
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<tr>
<td>$V,U,T,Q,\theta_A \theta_A$</td>
<td>1.01</td>
<td>—0.12</td>
<td>0.50</td>
<td>2.22</td>
<td>0.70</td>
<td>0.00</td>
<td>0.45</td>
<td>0.00</td>
<td>645.89</td>
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<tr>
<td>$V,U,T,Q$</td>
<td>1.00</td>
<td>—0.10</td>
<td>0.50</td>
<td>2.22</td>
<td>0.71</td>
<td>0.00</td>
<td>0.50</td>
<td>0.00</td>
<td>646.07**</td>
</tr>
</tbody>
</table>

*Most parsimonious models for untransformed (*) and transformed (**) data.
**Figures in italics indicate parameter fixed at value shown.
'No convergence to global maximum in mixed model. Highest likelihoods obtained at boundary with $H \rightarrow 0$.

(1.00 and 0.88, respectively, vs. 0.50) but not significantly, so, indicating limited power to test the Mendelian hypothesis with the family sample available. Thus, the most parsimonious model for the untransformed data was the additive mixed model with Mendelian transmission. The parameters for the most parsimonious model are plotted in Figure 1 (upper panel).

**Transformed data.** When skewness is present in the distribution of a quantitative trait, it is often considered appropriate to transform data to a normal distribution to guard against false inference of a major gene [Lalouel et al., 1983], although transformation also restricts power to detect major gene segregation [Demenais et al. 1986]. Thus, results are also presented for power transformed RBC HNMT activity.

The results from fitting various models to the transformed data are summarized in Table II. There was strong evidence for genetic transmission of RBC HNMT activity, since the model of no transmission, $(U,V)$, was rejected when compared with models allowing for familial transmission. The genetic transmission models were supported over the model of no transmission $(U,V)$ as follows: the polygenic model $(U,V,H, \chi^2 (1) = 30.38, P<10^{-7})$ and the Mendelian major gene model $(U,V,Q,T,D, \chi^2 (3) = 42.50, P<10^{-5})$. In the Mendelian mixed model $(U,V,D,Q,T,H)$, the parameters $(H)$ for polygenic transmission did not converge to the global maximum and the highest values were obtained as $H \rightarrow 0.00$. This boundary mixed model improved the fit over the polygenic model $(U,V,H, \chi^2 (3) = 12.12, P<0.01)$ but not over the Mendelian major gene model $(U,V,D,Q,T, \chi^2 (1) = 0.00)$. Thus, only major gene transmission was indicated. The Mendelian major gene model $(V,U,Q,T,D)$ did not differ from an additive model $(U,V,D = 0.5, Q,T, \chi^2 (1) = 0.57, \text{nonsignificant})$. Furthermore, the heterozygote transmission probability did not differ from Mendelian expectation (0.50) for either the full mixed model or the additive mixed model $(\chi^2 (1) = 0.22$ and 0.18,
Fig. 1. Segregation analysis of nuclear families: observed frequency distributions (bar graphs) of untransformed (upper panel) and power transformed (lower panel) RBC HNMT activity, both expressed in units/ml RBC. Expected component distributions (thin lines) for each HNMT activity genotype, and the total distribution (bold line), which is the sum of the three component genotypic distributions with age, gender, and generation effects removed. Expected genotypic means and distribution parameters are based upon major locus parameters from the Mendelian mixed model (Table II). Note in the upper panel that the right-most distribution for the high activity homozygotes is difficult to see because it accounts for only about 2% of the observations.

respectively, both nonsignificant). Furthermore, the heterozygote transmission probability differed little from its expected major gene model with Mendelian transmission. A graphic representation of the parameters from the best fitting Mendelian model is plotted in Figure 1 (lower panel).

DISCUSSION

Methylation is an important pathway in the metabolism of many drugs, xenobiotic compounds, and neurotransmitters [Weinshilboum, 1984, 1989]. HNMT catalyzes one of the two major pathways of histamine biotransformation in mammals, and, in the brain, it is thought to be the only significant pathway for the metabolism of this neuro-
transmitter compound [Schayer and Reilly, 1973; Schwartz et al., 1971]. HNMT, like several other methyltransferase enzymes, is present in an easily accessible human cell, the RBC [Axelrod and Cohen, 1971; Van Loon et al., 1985]. RBC levels of two other cytoplasmic methyltransferase enzymes, catechol O-methyltransferase (EC 2.1.1.6) and thiopurine methyltransferase (EC 2.1.1.67) are regulated by inheritance [Weinshilboum and Raymond, 1977; Weinshilboum and Sladek, 1980]. Furthermore, genetic variation of these enzyme activities in the human RBC reflects the relative levels of the same enzyme activities in less accessible organs or tissues [Weinshilboum, 1978; Woodson et al., 1982; Boudíková et al., 1990; and Szumlanski et al., 1992]. The presence of HNMT in the human RBC and the fact that we previously reported high family correlations for enzyme activity [Scott et al., 1988] made it important to study the possible role of genetic inheritance in the regulation of individual variation of HNMT activity in the human RBC.

In the present study we completed complex segregation analyses of HNMT activity in order to characterize the mode of inheritance of this important enzyme activity. We found evidence for major gene influence on the regulation of RBC HNMT activity. Analyses of the skewed untransformed data indicated the presence of a relatively rare \( Q = 0.121 \) additive major gene for high enzyme activity. Figure 1 shows that the three resulting genotypes had completely overlapping distributions accounting for 77, 21, and 2\% of individuals with low, intermediate, and high RBC HNMT activities, respectively. If this model is correct, then in the present sample of families, adults with RBC HNMT activity values above 240 units/ml RBC are very likely to be homozygous for a high HNMT activity allele, whereas those with RBC HNMT activity values below 90 units/ml RBC are very likely to be homozygous for a low HNMT activity allele.

Analyses of the normalized transformed data indicated the presence of a common \( Q = 0.67 \) additive major gene for high activity. Figure 1 shows that the three resulting genotypes had overlapping distributions accounting for 9, 41, and 50\% of individuals with low, intermediate, and high RBC HNMT activity, respectively. If this model is correct, then in the present sample of families, adults with RBC HNMT activity values above 190 units/ml RBC are very likely to be homozygous for a high HNMT activity allele, whereas those with RBC HNMT activity values below 70 units/ml RBC are very likely to be homozygous for a low HNMT activity allele.

Both transformed and untransformed data support the presence of Mendelian major gene segregation, but the gene frequency differences do not indicate a direct correspondence between genotypes inferred from the two sets of analyses. The analyses of transformed data give the best fit as well as the most parsimonious Mendelian major gene model. However, we cannot rule out the possibility of multiple alleles.

Isehuis et al.[1989] used the same methods and models employed in the present study to examine the utility of two-allele models in detecting segregation at a three-allele gene locus. They were able to detect multiple maxima at gene frequency values close to those identified as allele frequencies by electrophoretic studies. In the current analyses of the transformed data, we found only a single maximum for the most parsimonious model (at \( q = 0.71 \)). In analyses of the untransformed data we found two maxima for the most parsimonious model, but we obtained convergence only for the overall maximum (at \( q = 0.12 \)) reported in Table II, perhaps because the difference in likelihoods of the two maxima was large (-2 ln L = 22). The second, lower likelihood peak corresponded roughly to the gene frequency found for the transformed data (0.72).
Thus, there is some indirect supportive evidence for a three-allele locus with frequencies 0.33, 0.55, and 0.12, respectively, for the low, intermediate, and high activity alleles.

Quantitative segregation studies alone cannot unequivocally establish the presence of major gene segregation. However, the results of the present study do suggest a strategy for using RBC HNMT activity phenotypes to select individuals with a high probability of carrying particular RBC HNMT activity genotypes. In this regard the results for the analyses of the untransformed and transformed data may be combined. Molecular studies comparing structural and regulatory sequences of DNA from individuals with RBC HNMT activity values above 240 units/ml RBC and below 70 units/ml RBC are most likely to lead to the detection of molecular differences. These thresholds should be appropriate whether one follows results from the two-allele analysis of transformed or untransformed data and should be equally valid if HNMT activity were in fact determined by a three-allele locus. The resolution of this question will require molecular studies.

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