Hypotheses on the Effect of Cadmium on Glutathione Content of Red Blood Corpuscles

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Previous studies have shown that Glutathione, a tripeptide found in blood, is involved in protecting against toxins. Glutathione levels are known to drop in response to cadmium. Using 15 twin pairs, we modeled the effect of cadmium on glutathione levels. The heritability of glutathione content was 91%. The application of cadmium significantly reduced the mean level of GSH. However, this reduction in GSH was not due to additive genetic influences in our sample.

Glutathione (GSH), a tripeptide, is widely distributed in many animal cells and is known to participate in numerous cellular processes, particularly in protecting against, and removing, toxins. GSH is also involved in protein and DNA synthesis, maintenance of membrane integrity and regulation of enzyme activities (Meister, 1989; Meister & Anderson, 1983). Mukhopadhyay et al. (1988) has shown that GSH levels in the blood drop in reponse to cadmium (Cd2+). Lynn et al. (1997), investigating further, showed that GSH reduced the effect of cadmium to stop DNA strand breaks rejoining in Chinese hamster ovary cells. Here we determine the heritability of GSH in addition to testing several hypotheses surrounding the effect of cadmium on GSH. Heritability of enzymes important to the antioxidant defense system have already been published from this sample (Chakraborty & Das Chaudhuri, 2001). A heritability of .60 was found for Glutathione Peroxidase.

Data Collection and Statistical Methods

Blood samples were collected from 15 male twin pairs. Twins were selected for this study if they were between 14 and 40 years of age, taking no medication and raised together. Zygosity of twin pairs was established using serogenetic tests of ABO, Rh, MN and serum protein Hp. Additionally, the twins were gauged for similarity of appearance (Chakraborty & Das Chaudhuri, 2001; Siemens, 1924) at the Human Genetics Laboratory of the University of Calcutta (West Bengal, India). Seven of the twin pairs were monozygotic (MZ) and eight dizygotic (DZ). The mean age of the MZ and DZ twin pairs included in the study were 26 ± 5.9 years and 22 ± 3.5 years respectively. Consent was obtained from participants prior to participation in the study.

After removing the packed erythrocytes from each blood sample, these were suspended in 0.9% saline. Erythrocyte suspension from each sample was then divided into two equal parts by volume. One was incubated in 0.9% saline (representing control) and the other was in 0.1 nM $CdCl_2$ solution (representing treatment) at 37°C for 1 hour. GSH content (µmole/gHb) was then measured using DTNB for each part by measuring reflectance at 412 nm using a Hitachi U3210 UV-visible spectrophotometer (Chakraborty & Das Chaudhuri, 2001).

In addition to determining the heritability of GSH, genetic modeling can be used to decompose variation into genetic and environmental factors. Specifically, it is possible to estimate an additive genetic effect (A, or σ^2_γ, its variance), a common environmental effect (C), a nonadditive genetic effect (D) and a unique environmental effect (E, or σ^2_ε, its variance). Additive genetic influences are those where the effect on the trait is the sum of the effects of the alleles influencing the trait. Environmental influences are said to be unique when they influence only one twin in a pair. The unique environmental influence, E, includes measurement error. Here we estimate only additive genetic and unique environmental sources of variation (AE model) due to limited power from the small sample size. To address pertinent hypotheses, the data was modeled as a Cholesky decomposition where variation in GSH levels after cadmium treatment are decomposed into additive genetic and unique environmental influences common with GSH levels before treatment and additive genetic and environmental

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Table 1

Results of Hypothesis Testing

Tests of heterogeneity	Model fit	*df*	$\Delta\chi^2$	Δ *df*	*p* value
Saturated model	420.95	52			
Test 1. Test unique genetic influence on GSH due to cadmium treatment	420.95	53	0.00	1	1.000
Test 2. Does the genetic influence on GSH before treatment equally influence GSH after treatment?	424.22	54	3.27	1	.070
Test 3. Does the environmental influence on GSH before treatment influence GSH after treatment?	**425.83**	**55**	**1.61**	**1**	**.205**
Test 4. Difference in mean GSH level before and after cadmium treatment?	505.24	56	79.41	1	.000

Note: Most parsimonious model in bold.

influences unique to GSH levels after treatment (for more detail on genetic modeling, see Neale & Cardon, 1992; Posthuma et al., 2003).

Sources of variation and means were estimated using the computer program Mx (v1.57a, Neale et al., 2002). Nested models were run to test hypotheses and determine the most parsimonious model to explain the data. Twice the difference in log likelihoods between the full and submodels is distributed as chi-squared with the degrees of freedom equal to the difference in degrees of freedom between the two models (likelihood ratio test, Neale et al., 1992).

Results and Discussion

A path diagram showing the maximum likelihood estimates of all parameters prior to fixing the value of particular paths to zero is shown in Figure 1. Genetic influences on GSH after cadmium treatment could be explained by the genetic influences on GSH before treatment (Test 1, Table 1). Although this suggests that the effect of cadmium in reducing GSH levels is not due to genetic influences, there is little power in this study to detect such an influence, particularly if the magnitude of the effect is small. The proportion of variance in GSH due to additive genetic influences can be estimated equal before and after treatment with cadmium (Test 2, Table 1). A model could not be fitted where a single environmental influence explained uncorrelated variance in both GSH content before and after treatment with cadmium. There were no unique environmental influences common to GSH levels both before and after treatment (Test 3, Table 1). This suggests that the

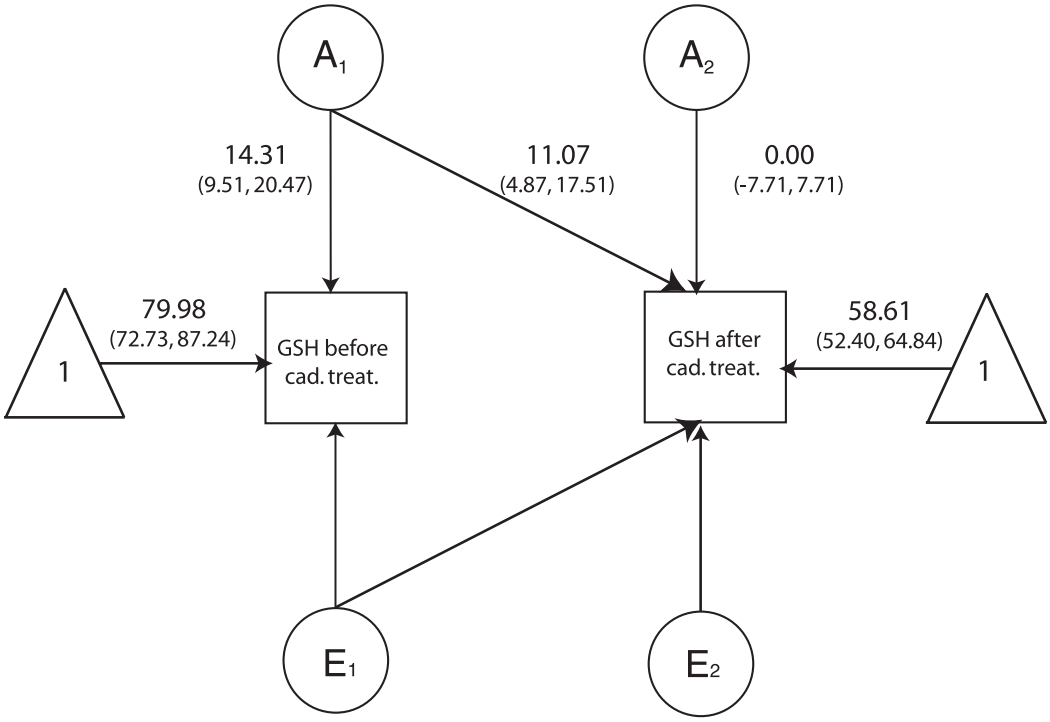

Figure 1

Maximum likelihood estimates of parameters explaining GSH content before and after cadmium treatment.

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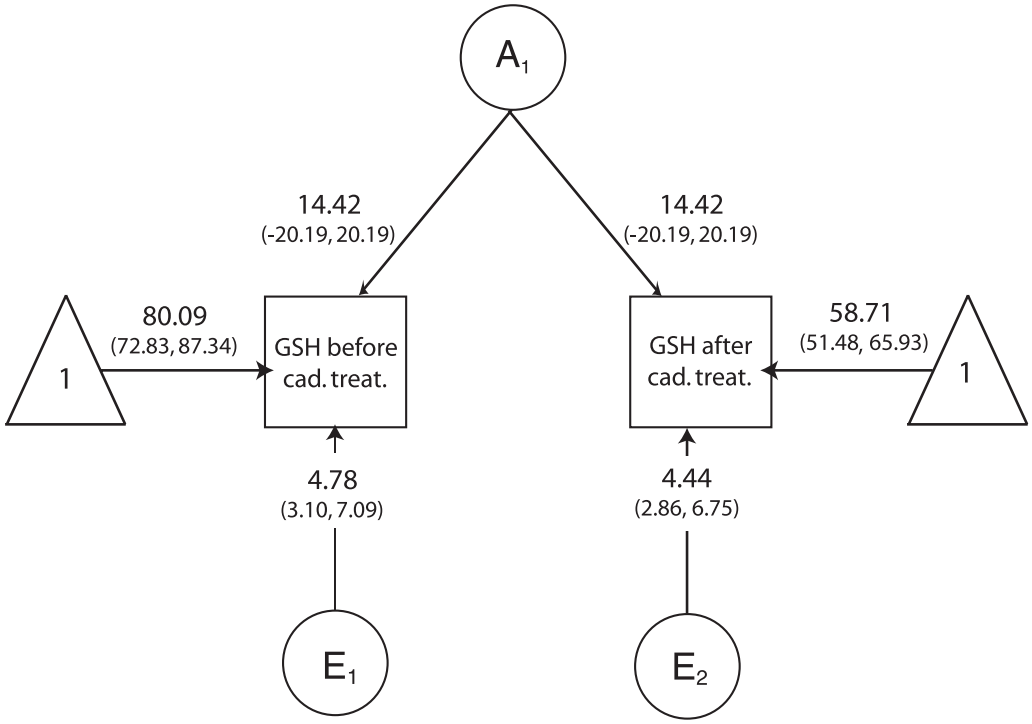

Figure 2

Most parsimonious model to explain means, variances and covariances in GSH content before and after treatment with cadmium.

estimated unique environmental influences may be due to measurement error. The result of Test 4 (Table 1, triangles in Figure 2) indicates that there was a significant reduction in GSH levels with cadmium treatment, as reported previously by Mukhopadhyay et al. (1988). Additive genetic effects could not be removed as a source of variation without a significant drop in fit of the model (χ^2_1 = 390). The model to best explain the data is illustrated in Figure 2, suggesting a heritability for GSH of 91%. A more definitive study of the effect of cadmium on glutathione requires a study with a larger sample size.

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