

# Acetylation Phenotype in Abstinent Alcoholics

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**No association between acetylation phenotype and alcoholism was discovered. Fifty-four percent of both the alcoholic patients and healthy volunteers were rapid acetylators. Acetylation phenotyping is not helpful to the investigation of the genetics of alcoholism.**

**T**HERE HAS BEEN an accumulation of data suggesting that vulnerability to alcoholism is markedly influenced by genetic factors. Strongest evidence for this has come from studies in which a higher level of concordance for alcoholism was found among identical than fraternal twins,<sup>1,2</sup> and studies of adopted offspring of alcoholics.<sup>3-6</sup> The identification of a genetic marker for alcoholism would narrow the search for a "vulnerability gene." Several blood proteins have been reported to differ in alcoholics when compared to controls,<sup>7</sup> and platelet MAO and adenylate cyclase activity have been noted to be low in alcoholics.<sup>8-10</sup> However, it is unclear whether or not these apparent changes predate the development of alcoholism. A biological marker that correlates with a genetic vulnerability towards alcoholism has not been identified. One approach is to compare the occurrence of alcoholism to the expression of a readily identifiable phenotype that is known to be the result of two alleles at a autosomal gene locus, as is the acetylation phenotype.<sup>11,12</sup>

Other reasons for determination of any cosegregation of alcoholism with the rapid or slow acetylation phenotype have to do with a reported increase in acetylation clearance of drugs when administered with alcohol<sup>11,13</sup> and the metabolism of serotonin by *N*-acetyltransferase. Serotonin has been hypothesized to exhibit abnormal metabolism in patients with alcoholism.<sup>14</sup> It has been reported that the cerebrospinal fluid concentrations of the serotonin metabolite, 5-hydroxyindole acetic acid, are low in subjects who display pathological impulsive behavior.<sup>15-18</sup> Also, personality traits associated with impulsive behavior have been ascribed to Type 2 alcoholics, who exhibit a strong family history of paternal alcoholism.<sup>14</sup>

## METHODS

Acetylation phenotype was established in 63 subjects: 37 abstinent alcoholics and 26 control subjects. All subjects were Caucasian, 18 years

of age or older and unrelated to any other volunteer. None exhibited any abnormalities on physical screening, which included a history and physical examination, complete blood count with differential, platelet count, liver enzymes, albumin, BUN, creatinine, and urinalysis. Although liver biopsy was not performed, any alcoholic subject with abnormal liver enzyme values was excluded. Volunteers and alcoholic subjects were drug-free, with the exception of two controls; one taking conjugated estrogens and the other atenolol. All subjects with a history of allergy to sulfa drugs or glucose-6-phosphate dehydrogenase deficiency were excluded. Alcoholic patients were diagnosed as alcohol dependent according to DSM-III<sup>19</sup> criteria based on a structured interview using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version.<sup>20</sup> Control subjects were screened with these same instruments and were admitted into the study only if they were free of any drinking problems and had no alcoholic first degree relative. All abstinent alcoholics and some controls stayed on an inpatient ward located at the National Institute of Health Clinical Center in Bethesda, MD. The alcoholic subjects had all been abstinent for at least 1 month prior to participation in the study. The remainder of the control subjects participated in the study as outpatients. All controls were asked to abstain from drinking any alcoholic beverage for 48 hr prior to the acetylator phenotype testing. Compliance was tested with a breath alcohol test which was required to be negative prior to beginning the acetylation phenotyping procedure. All subjects signed an informed consent form prior to participating in the study and control subjects were financially reimbursed.

There were 26 controls and 37 abstinent alcoholics. There was no significant difference in age between the two groups (controls:  $49 \pm 15$  years vs. alcoholics:  $46 \pm 20$  years). There was a higher proportion of women in the control group (11 of 26 vs. 4 of 37).

All subjects fasted from midnight on the day of the study, except for water, and received a 20-mg/kg oral dose of sodium sulfamethazine solution in the morning between 6:45 and 8 a.m. The subjects then continued fasting, with the exception of water, for an additional 3 hr. Ten milliliters of blood were drawn from an antecubital vein into a vacutainer tube at 5 hr following sulfamethazine administration.

Sulfamethazine serum samples were assayed using high-performance liquid chromatography with UV detection employing the method of Whelpton et al.<sup>21</sup> The concentrations of both sulfamethazine (SMZ) and acetylsulfamethazine (ACSMZ) were determined directly by this method. The percentage acetylated at 5 hr was calculated as follows (concentrations are expressed as mcg/ml)<sup>22</sup>:

$$\frac{[\text{ACSMZ}]}{[\text{ACSMZ}] + [\text{SMZ}]} \times 100 = \% \text{ of total concentration acetylated}$$

## RESULTS

The proportions in the two groups were compared using a  $\chi^2$  test. They did not differ from each other. The percentage acetylated at 5 hr showed a bimodal distribution in both groups (Figs. 1 and 2). The cut-off point between fast and slow acetylators was set at 60%, a posteriori. The alcoholic and control groups contained, respectively, 54% (20/37) and 54% (14/26) fast acetylators. Eleven of the male alcoholic patients had a family history of alcoholism based on two first degree male relatives (father and either

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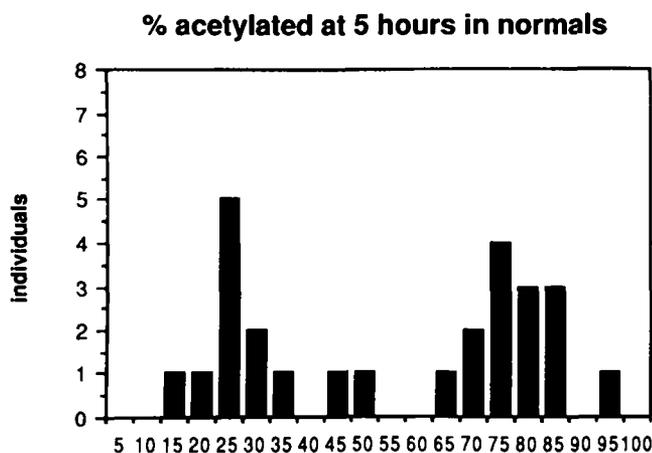


Fig. 1. The percentage of plasma sulfamethazine acetylated at 5 hr following oral administration to 26 control subjects.

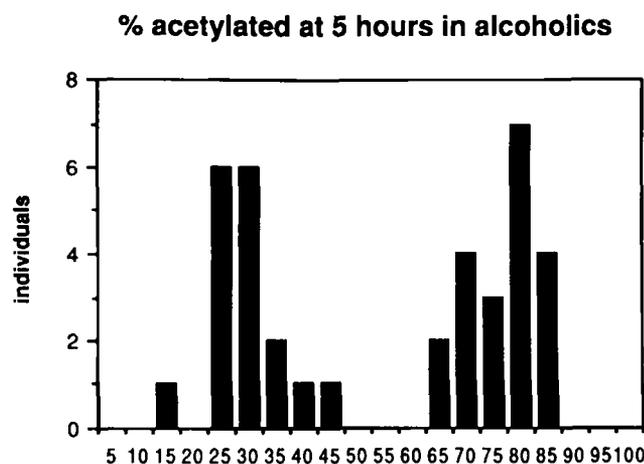


Fig. 2. The percentage of plasma sulfamethazine acetylated at 5 hr following oral administration to 37 abstinent alcoholics.

paternal grandfather, brother or son). Six of them were rapid and five were slow acetylators of sulfamethazine, which is no different from the proportion in the control subjects.

No side-effects to the sulfamethazine were noted, with the exception of one complaint of a headache.

#### DISCUSSION

Our study revealed no difference in the proportions of slow or fast acetylators in abstinent alcoholics when compared to normal volunteers.

Acetylator phenotype is genetically determined by two autosomal alleles and governs the metabolic rates of many drugs undergoing acetylation. Although three genotypes (fast, moderate, slow) exist for acetylation,<sup>23</sup> most commonly used techniques for determining acetylation phenotype only discriminate between "fast" and "slow" acetylators. Intermediate acetylators are included with fast acetylators when abbreviated tests, such as the one used in this study, are used. Acetylation rate may have a strong bearing on drug efficacy and also upon the presence of

certain side-effects, especially when an acetylation step is the major activating or deactivating step in the metabolic pathway, e.g., isoniazid and hydralazine. Additionally, the slow acetylators show a greater incidence of arylamine-induced bladder cancer, since they detoxify arylamines more slowly than fast acetylators.<sup>24,25</sup> Two studies<sup>26,27</sup> have also found an association between acetylator phenotype and personality traits. These personality traits (neuroticism and hypochondriasis) cannot be simply related to those ascribed to Type 1 and Type 2 alcoholics as described by Cloninger.<sup>14</sup>

In the initial acetylation phenotyping studies,<sup>25,28</sup> acetylation phenotype appeared to be uninfluenced by either age or gender. However, recent studies report that both gender and age affect isoniazid acetylation rate.<sup>29,30</sup> Gachalyi et al.<sup>31</sup> have also reported that age affects acetylator phenotype when it is determined using sulfamethazine. There was no significant difference in age between our two groups. Since gender has not been shown to be a significant source of variability when sulfamethazine is used as a phenotyping agent the greater number of females in our control group most likely had no effect on the results.

Our phenotype results (54% fast, and 46% slow in each group) are in general agreement to those reported for the caucasian population, 52% fast and 48% slow.<sup>32</sup> A power analysis based on our current results reveals that, should there be a significant difference ( $\alpha = 0.05$ ,  $Z_{\alpha} = 1.96$ ) between the two groups, a sample size of 2430 subjects would be required in each group to demonstrate this difference with a power of 80% ( $1 - \beta = 0.8$ ,  $Z_{\beta} = 0.84$ ). Based upon this information a significant association between acetylation phenotype and the presence of alcohol abuse or dependence is very unlikely.

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