Rate of Caffeine Metabolism and Risk of Spontaneous Abortion

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In a case-control study of 73 women with and 141 women without spontaneous abortion, the authors determined the activity of the three principal caffeine-metabolizing enzymes—cytochrome P-4501A2 (CYP1A2), xanthine oxidase, and N-acetyltransferase 2—by measuring levels of caffeine metabolites in urine. After examining the effect of enzyme activity and different levels of caffeine intake, they concluded that there was no evidence that an interaction between enzyme activity and caffeine intake during pregnancy resulted in risk of spontaneous abortion. In a subsample comparing 24 cases with recurrent (two or more) spontaneous abortions and 21 controls with two or more livebirths and no previous spontaneous abortions, the unadjusted odds ratio for low CYP1A2 enzyme activity (below the median) was 0.92 (95% confidence interval (CI) 0.28–3.04) compared with higher CYP1A2 activity. The odds ratio for risk of recurrent spontaneous abortion and low xanthine oxidase activity (below the median) versus higher activity was 0.37 (95% CI 0.10–1.29). Phenotypically slow acetylators (N-acetyltransferase 2 index <0.37) had an odds ratio of 1.58 (95% CI 0.48–5.13) for recurrent loss compared with rapid acetylators. Thus, some association of the latter two caffeine-metabolizing enzymes with recurrent spontaneous abortion is suggested but may also be due to chance. Am J Epidemiol 1998;147:503-10.

Differences in rates of metabolism play an important role in sensitivity to a number of drugs and also in affecting susceptibility to injury after exposure to environmental toxicants (1–4). Results from previous studies have suggested that caffeine consumption may be related to risk for spontaneous abortion (5). Although our own most recent study did not demonstrate any relation of caffeine consumption to spontaneous abortion (6), this finding may have been attributable to different rates of caffeine metabolism among women in the study. Therefore, we examined subsets of women defined by their ability to metabolize caffeine. The level of caffeine in the body depends on the amount of caffeine consumed and the rate at which caffeine is eliminated from the body. Individuals vary widely—up to 10-fold—in rates of caffeine metabolism (7). It is possible that such metabolic differences influence susceptibility to adverse reproductive effects of exogenous exposures by influencing the effective dose or extent of exposure to pharmacologically active metabolites (8).

The rate of caffeine metabolism in humans is determined primarily by the activity of cytochrome P-4501A2 (CYP1A2) (9). Caffeine is metabolized to a lesser extent by xanthine oxidase and N-acetyltransferase 2 (10). CYP1A2 is inducible by polycyclic aromatic hydrocarbons and converts precarcinogens into carcinogens (7, 9, 10). Although xanthine oxidase and N-acetyltransferase 2 play only a minor role in the metabolism of caffeine, they have potential importance as markers of various toxicologic phenomena. For example, xanthine oxidase is a source of active oxygen species that may be important in aging and carcinogenicity (10). Genetically low activity of N-acetyltransferase 2 has been associated with an increased risk of toxicity to certain drugs and an increased risk of aryamine-produced bladder cancers (10). A small case-control study found an elevated but nonsignificant risk for recurrent spontaneous abortion and slow N-acetyltransferase 2 genotype (11). In our study, we did not perform a genetic analysis of drug-metabolizing enzymes, but, rather, we measured phenotypic activity by measuring caffeine metabolite ratios in urine after a test dose of caffeine (12, 13).

Both the rate of caffeine clearance and the in vivo activity of caffeine-metabolizing enzymes can be estimated by measuring caffeine metabolites in the urine.
after a caffeine challenge protocol as described below (12, 13). This test provides a noninvasive way to study the relation between drug-metabolizing enzyme activity and disease in large populations. The first objective of this study was to examine the possibility that slower metabolizers of caffeine (that is, persons with lower indices of CYP1A2 activity) are more susceptible to an effect of caffeine on spontaneous abortion. Differential activity of one or more of the enzymes, if associated with risk of spontaneous abortion, may also represent a marker for metabolism of an agent or agents other than caffeine. Therefore, a second objective of this study was to examine whether the risk for spontaneous abortion was associated with differential activity of one or more of the three caffeine-metabolizing enzymes. A third objective was to investigate whether the risk of recurrent spontaneous abortion (defined as two or more) was associated with differential activity of any of these three enzymes.

MATERIALS AND METHODS

Subjects

A sample of women who had spontaneous abortions (cases) and a sample of women who delivered live-births and stillbirths (controls) were selected from a previously conducted study of pregnant women (Pregnancy Outcome Study) and were invited to participate in a study to determine their rate of caffeine metabolism. The Pregnancy Outcome Study, which has been described previously (14), was a prospective study designed to examine primarily the associations between several environmental factors and spontaneous abortion. Briefly, the study population included 5,342 pregnant women recruited from Kaiser Permanente Medical Care Program, a large prepaid health plan, at the time they scheduled their first prenatal appointment. Women were interviewed on average at approximately 8 ± 2 weeks gestational age. Pregnancy outcomes were obtained for 99 percent of the interviewed women.

Women from the Pregnancy Outcome Study whose pregnancy ended at ≤20 completed weeks of gestation were defined as spontaneous abortions (cases). All women with livebirths and women with stillbirths (losses >20 weeks gestational age) were defined as controls. For logistical and budgetary reasons, we restricted recruitment for the caffeine metabolism study to participants from Santa Clara County and Walnut Creek/Antioch in northern California; the original study also included Kaiser members from southern California. We attempted to recruit all cases from northern California who reported consuming caffeine either before or during pregnancy. We randomly selected controls for recruitment within broad categories of caffeine consumption reported in the Pregnancy Outcome Study, and we tried to frequency match two controls for each case enrolled.

Of the 445 women who were contacted for this study, 121 declined to participate and thus were not screened for eligibility (described below). The percentages of cases and controls refusing were similar. A total of 102 women who agreed to participate were ineligible for the metabolism study due to one of the following conditions that can affect caffeine metabolism (7, 15, 16): current use of oral contraceptives, currently pregnant, use of medications such as theophylline, or diagnosis of specific medical conditions such as chronic liver disease.

A total of 222 women participated in the caffeine metabolism study. Exclusions were made due to excessively low caffeine metabolite levels measured after the test dose of caffeine in seven women, marginal metabolite levels in three women, and loss of one woman’s samples, leaving 211 samples available for analyses. One subject (of the 211) had a usable value for N-acetyltransferase 2 but unusable CYP1A2 and xanthine oxidase values. Among the 211 women, 72 cases were classes and 139 were controls (136 live-births and three stillbirths).

Laboratory methods

We used the caffeine test protocol described by Kalow and Tang (7). Subjects were asked to refrain from all caffeinated beverages, chocolate, and alcohol for 2 days before and until the time of urine collection. At about 4:00 p.m. of the test day, subjects were asked to consume a capsule containing 200 mg of caffeine. The next morning, the first-voided urine was collected for analysis. Women were asked to provide a saliva sample before consuming the caffeine capsule to determine compliance with caffeine abstention. The saliva sample was assayed for caffeine by gas chromatography, using a capillary column with nitrogen-phosphorus detection and 7-ethyl-theophylline as the internal standard, as described by Shi and co-researchers (17). Salivary levels are known to be highly correlated with plasma levels of caffeine (18).

Concentrations of various caffeine metabolites in urine, which can be used to estimate caffeine clearance, can be measured in urine by high performance liquid chromatography (12, 13). Assays for these metabolites were performed in the Clinical Pharmacology Laboratory at the San Francisco General Hospital/University of California, San Francisco, using the methods described by Tang-Liu and Riegelman (19) and Tang et al. (20). Metabolic indices for CYP1A2, xanthine oxidase, and N-acetyltransferase 2 were cal-
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Calculated using urine caffeine metabolite ratios according to the methods described by Kalow and Tang (9) and Tang and collaborators (21). The specific ratios used were as follows:

\[
\text{CYP1A2 index} = \frac{[\text{AAMU}] + [1U] + [1X]}{[17U]},
\]

\[
\text{N-acetyltransferase 2 index} = \frac{[\text{AAMU}]}{[1U] + [1X]},
\]

\[
\text{Xanthine oxidase index} = \frac{[1U]}{[1U] + [1X]},
\]

where

\[
1U = 1\text{-methyluric acid},
\]

\[
1X = 1\text{-methylxanthine},
\]

\[
17U = 1,7\text{-dimethyluric acid},
\]

\[
\text{AAMU} = 5\text{-acetylamino-6-amino-3-methyluracil}.
\]

Questionnaires

Caffeine metabolism questionnaire. At the time of the caffeine metabolism test, information was collected in a face-to-face interview on the following factors, which could be related to enzyme activity: age, race, current smoking status, typical passive smoke exposure at home, charbroiled food consumption in the previous 2 weeks, and medications regularly taken.

Pregnancy Outcome Study questionnaire. The original study interview was administered over the phone in the first trimester during 1990–1991. Data that were collected included date of last menstrual period, demographics, reproductive and medical history, and consumption of alcohol, tobacco, and caffeinated beverages. Subjects were asked to quantify (cups or cans per day) their daily consumption of caffeinated beverages (coffee, tea, and soda) in the week before the interview (hereafter referred to as “during pregnancy” or “during the first trimester”). The same questions were asked with reference to the week around their last menstrual period (hereafter referred to as “before pregnancy”). We calculated total caffeine consumption by assuming a caffeine content of 107 mg/cup of coffee, 34 mg/cup of tea, and 47 mg/can of soda (22).

Statistical analyses

Our overall data analysis strategy consisted of two steps. We first identified predictors of enzyme activity in our data because these predictors could be potential confounders of any relation between spontaneous abortion and measures of enzyme activity and caffeine intake. We then used these results in combination with those from the literature (23) to select covariates to model the risk of spontaneous abortion by each index of enzyme activity for different levels of caffeine intake. Before step one, univariate distributions of the urine metabolite indices were visually examined to determine whether they were normally distributed. The variable reflecting CYP1A2 activity was normalized by using a logarithmic transformation (base 10).

The distribution for N-acetyltransferase 2 activity appeared to be bimodal, as expected from previous research (21). It was determined that those subjects with an N-acetyltransferase 2 index <0.37 formed one cluster (n = 114), denoted as “slow acetylators”; and those subjects with an N-acetyltransferase 2 index >0.37 (n = 97) formed the second cluster, designated as “rapid acetylators.” This breakpoint for classifying subjects as rapid or slow acetylators was determined using model-based hierarchical clustering (24).

The means of the indices that reflect enzyme activity were compared using t tests or analysis of variance across variables suspected of being related to enzyme activity from previous research. We fit separate multiple linear regression models with each of the measures of enzyme activity as the dependent variable and the variables suspected to influence enzyme activity as the independent variables. The association of the following variables were assessed: smoking status, passive smoke exposure at home, charbroiled food consumption, race, age, and medications that were regularly taken. Medications were divided into three categories: progesterone, thyroid medications, and “other medications.”

To determine whether slower caffeine metabolism might affect a woman’s susceptibility to any effects of caffeine consumption on risk for spontaneous abortion, we performed logistic regression (25, 26) and included an interaction term (27) between the enzyme variable (slow/rapid) and caffeine intake during pregnancy. We entered caffeine as a categorical variable in the models with the following levels of consumption: no caffeine, 1–150 mg of caffeine per day, and >150 mg of caffeine per day. Small numbers of women consuming >300 mg per day (n = 10) limited our ability to look at this category. Xanthine oxidase activity and the log of CYP1A2 activity were modeled both as continuous predictors and as binary variables using the median as the cutpoint. We found no appre-
ciable differences in results comparing the 25th and 75th percentiles, instead of the median, so we chose to present results using the median as the cutpoint. N-acetyltransferase 2 activity was modeled as a dichotomous variable with the high-risk women being the slow acetylators. We calculated both unadjusted and adjusted odds ratios. We adjusted for maternal age, pregnancy history, smoking, and alcohol consumption during pregnancy in the model, exploring the activity of CYP1A2 in relation to spontaneous abortion. In addition to the covariates listed above, we included race in the models, examining the activity of xanthine oxidase and N-acetyltransferase 2 in relation to spontaneous abortion. We also used logistic regression to examine the relation between each index of enzyme activity and spontaneous abortion. We performed these analyses both without and with the inclusion of the covariates noted above.

For each of the final models, we ran one analysis excluding the six women who had high levels of caffeine in their saliva at the time of the caffeine challenge test (>400 ng/ml) to determine whether their removal affected our results. We also ran each final model excluding the women with stillbirths (n = 3). Neither appreciably changed any of the results. In addition, we selected a nested group of cases and controls, defining cases as women with a history of two or more spontaneous abortions (n = 24) and controls as having had two or more livebirths and no previous spontaneous abortions (n = 21). We calculated the crude odds ratio for risk of recurrent spontaneous abortion for the slow acetylators compared with the rapid acetylators, as well as for low CYP1A2 enzyme activity (below the median) and low xanthine oxidase activity (below the median). The small numbers in this subset analysis did not allow us to estimate reliably the risk for recurrent spontaneous abortion in relation to caffeine intake and enzyme activity.

RESULTS

The mean age of the participants was 30 years, and 80 percent of the subjects were white. In table 1, a comparison of the characteristics of participants and women who refused to participate in the caffeine metabolism study is presented. Women who declined to participate in the study were more likely to be of lower socioeconomic status, to have been interviewed in the original study at a later gestational age of their pregnancy, and to be nonwhite. There was little difference between participants and refusers for reported caffeine consumption during pregnancy. Approximately 34 percent of the women did not consume any caffeine during pregnancy, and almost 5 percent consumed >300 mg/day. At the time of the caffeine metabolism test, about 19 percent of the participants reported smoking; whereas earlier, during pregnancy, approximately 12 percent reported smoking.

The median, mean, standard deviation, and range for each of the caffeine metabolite indices reflecting enzymatic activity are presented in table 2. Initially, the results of the CYP1A2 regression model suggested an association between the category “other medication” and the index of CYP1A2 activity; subjects in the other medication category had lower CYP1A2 indices. Evidence exists that propanolol, erythromycin, conjugated estrogens, cimetidine, and verapamil all might influence the CYP1A2 activity (14). Therefore, one model was run removing the subjects who used those drugs (n = 6), and the category of other medications was no longer related to CYP1A2 activity. For all subsequent models involving CYP1A2 activity, we excluded these six women. Smoking status remained the only covariate that was associated with CYP1A2 activity in the regression model. On the log scale, the CYP1A2 index for nonsmokers (n = 163) was 0.76 and for smokers (n = 41) was 1.02 (p < 0.0001).

For 24 women, the reported smoking behavior at the time of the metabolism test was not the same as the reported smoking behavior during pregnancy. Because smoking was so strongly related to CYP1A2 activity, for all subsequent analyses of CYP1A2 we attempted to prevent misclassification of these women’s CYP1A2 indices (as related to pregnancy) in the following two ways. First, we ran one analysis deleting the women (n = 24). Second, we imputed the CYP1A2 indices for these women based on their reported behaviors during pregnancy. Thus, for the 20 women who did not smoke during pregnancy but did report smoking at the time of the metabolism test, we subtracted 0.26 from the log scale (the difference noted above between smokers and nonsmokers for CYP1A2 activity) and we added 0.26 for the converse situation. We found no appreciable difference in results for all subsequent CYP1A2 analyses using these two methods; we chose to present results with these women included using the imputed indices.

Both race and age appeared to be related to xanthine oxidase activity. Asian/Pacific Islanders had lower levels of xanthine oxidase activity relative to whites, and the confidence intervals did not overlap. The least squares means and 95 percent confidence intervals were 0.63 (0.62–0.64) for whites, 0.61 (0.59–0.64) for Hispanics, 0.59 (0.54–0.65) for blacks, and 0.58 (0.56–0.61) for Asian/Pacific Islanders. We also observed that the activity of xanthine oxidase increased with age. Age (modeled as a continuous covariate) produced an estimated coefficient of 0.002 (p = 0.01). Thus, for an age difference of 10 years (holding race
TABLE 1. Comparison of demographic characteristics between subjects participating and those who refused, California, 1992–1994

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Metabolism subjects (n = 211; % = 63.5)</th>
<th>Refusers (n = 121; % = 36.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤32</td>
<td>143</td>
<td>87</td>
</tr>
<tr>
<td>&gt;32</td>
<td>68</td>
<td>34</td>
</tr>
<tr>
<td>Gestational age at interview (weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤8</td>
<td>161</td>
<td>79</td>
</tr>
<tr>
<td>&gt;8</td>
<td>50</td>
<td>42</td>
</tr>
<tr>
<td>Pregnancy history (prior losses)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>194</td>
<td>114</td>
</tr>
<tr>
<td>≥2</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married or living together</td>
<td>207</td>
<td>119</td>
</tr>
<tr>
<td>Separated/divorced/widowed</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>169</td>
<td>77</td>
</tr>
<tr>
<td>Black</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Asian</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Hispanic</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Alcohol consumption during pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>None</td>
<td>187</td>
<td>108</td>
</tr>
<tr>
<td>Cigarette consumption during pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>None</td>
<td>185</td>
<td>101</td>
</tr>
<tr>
<td>Socioeconomic Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low income and low education</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Others</td>
<td>195</td>
<td>99</td>
</tr>
<tr>
<td>Caffeine consumption (mg/day)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>72</td>
<td>36</td>
</tr>
<tr>
<td>1–150</td>
<td>93</td>
<td>55</td>
</tr>
<tr>
<td>151–300</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td>&gt;300</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

* Conversion to mg of caffeine during pregnancy was based on 107 mg/cup of coffee, 34 mg/cup of tea, and 47 mg/can of soda (Bunker and McWilliams, 1979 (22)).


<table>
<thead>
<tr>
<th>Enzyme variable (transformation)</th>
<th>No.</th>
<th>Median</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2* (log10)</td>
<td>204</td>
<td>0.79</td>
<td>0.81</td>
<td>0.19</td>
<td>0.36–1.59</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>210</td>
<td>0.63</td>
<td>0.62</td>
<td>0.06</td>
<td>0.31–0.78</td>
</tr>
<tr>
<td>Slow acetylators†</td>
<td>114</td>
<td>0.27</td>
<td>0.27</td>
<td>0.05</td>
<td>0.14–0.36</td>
</tr>
<tr>
<td>Rapid acetylators‡</td>
<td>97</td>
<td>0.53</td>
<td>0.53</td>
<td>0.09</td>
<td>0.38–0.73</td>
</tr>
</tbody>
</table>

* CYP1A2, cytochrome P-4501A2.
† Slow acetylator defined by a N-acetyltransferase 2 index <0.37.
‡ Rapid acetylator defined by a N-acetyltransferase 2 index >0.37.

constant), we would predict a xanthine oxidase index difference of 0.02.

Among the rapid acetylators only, the N-acetyltransferase 2 indices of Asians and blacks were higher than for whites and Hispanics. The least squares means and 95 percent confidence intervals by race were 0.60 (0.50–0.69) for blacks, 0.59 (0.54–0.64) for Asian/Pacific Islanders, 0.52 (0.50–0.54) for whites, and 0.51 (0.45–0.57) for Hispanics. None of the covariates were related to the index of N-acetyltransferase 2 in the model restricted to slow acetylators.

In table 3, the crude and adjusted effect estimates for spontaneous abortion by the indices of enzyme activity (lower versus higher) for different levels of caffeine intake are presented. The measures of association do not reveal a consistent pattern between increasing dose and risk among women with lower indices of enzyme activity. Adjustment resulted in some difference in the effect estimates, but that does not change our interpretation of the results. We did not find any evidence for the presence of an interaction between enzyme activity (CYP1A2, N-acetyltransferase 2, xanthine oxidase)
justed models were 0.41, 0.49, and 0.32, respectively; the confidence limit includes unity. In contrast, the association for risk of recurrent spontaneous abortion, but again the confidence limit is very wide and includes one.

DISCUSSION

To our knowledge, this is the first study to examine the relation between rate of caffeine metabolism, caffeine intake, and risk of spontaneous abortion. To estimate the rate of caffeine metabolism, we used urine caffeine metabolite ratios (CYP1A2 index), which have been shown to predict the clearance (rate of metabolism) of caffeine (13). For our purposes, phenotyping, which describes the actual in vivo enzyme activity, is more useful than genotyping. Genotype alone provides an incomplete picture because genotype and environment interact to determine in vivo activity. We did not observe a consistent pattern of dose-related increased risk for spontaneous abortion among women who metabolized caffeine more slowly. Slower enzyme activity did not appear to be related to risk of spontaneous abortion overall.

Our effect estimate and wide confidence interval for the risk for recurrent spontaneous abortion and slow N-acetyltransferase 2 phenotypic activity are similar to those reported by Hirvonen and coworkers (11). Comparing slow versus rapid N-acetyltransferase 2 genotype using deoxyribonucleic acid analysis, the race-adjusted Mantel-Haenszel odds ratio for recurrent loss in that study was 1.4 (95 percent confidence interval (CI) 0.45–4.3) using clinic controls and 1.6 (95 percent CI 0.69–3.7) using community controls (11). Given the wide confidence intervals reported in both studies, there is not enough information to either support or rule out an effect. This is also true for the lowered effect estimate seen in our study for low xanthine oxidase activity and recurrent spontaneous abortion.

Some limitations of our study should be noted. We did not measure enzyme activity during pregnancy; we assumed that the relative measures of enzyme activity (with the exception of women with discrepant smoking behaviors) remained the same after pregnancy as during the first trimester. This assumption is reasonable because although the hormonal changes of preg-
nancy are known to slow the rate of metabolism of caffeine as early as the first half of pregnancy, they occur predominately in the third trimester (16). Another limitation is that we had only 10 women who reported consuming >300 mg caffeine per day during pregnancy. However, results from a univariate analysis of the interaction of slow metabolism and caffeine consumption in these 10 women were not appreciably different from those in women consuming >150 mg of caffeine. Finally, because of small numbers, we were not able to reliably estimate the risk for recurrent spontaneous abortion in relation to caffeine consumption and the indices of enzyme activity.

Our observations of the associations between enzyme activity with smoking and ethnicity are in general agreement with prior research. Caffeine clearance as indicated by CYP1A2 has previously been shown to be accelerated in smokers (7, 10). Asian populations are known to have a higher prevalence of fast acetylators compared with whites (28). In contrast to our finding, Relling and collaborators (29) did not find a relation between age and xanthine oxidase. However, their study population comprised males and females, was 20 percent black, and included no Asian subjects.

Our results showed no association between caffeine consumption, caffeine metabolism, and risk of spontaneous abortion. However, our findings suggest that further study of the relation between recurrent spontaneous abortion and acetylator status and xanthine oxidase activity should be considered in larger studies. Using a one-to-two case-control sampling scheme, the sample size necessary to achieve 80 percent power (significance at the 0.05 level), given an odds ratio of 1.58 for slow acetylator status, is 191 cases of recurrent spontaneous abortion and 382 controls. Using the same criteria, assuming an odds ratio of 0.37 for recurrent abortion and low xanthine oxidase activity, one would need 40 cases and 80 controls.

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