Do GST Polymorphisms Modulate the Frequency of Chromosomal Aberrations in Healthy Subjects?

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Rossi et al. (2009) described an association between chromosomal aberration (CA) frequency and cancer risk in a case–control study on 107 cancer cases and 291 controls, whereby they observed no modifying effect of polymorphisms in glutathione S-transferase M1 (GSTM1) and GSTT1.

In our studies of 488 healthy individuals who shared the same environmental exposure in Slovakia and the Czech Republic, we observed a CA frequency of 2.35 ± 1.73 (mean ± SD) (Halasova et al. 2007; Musak et al. 2008; Naccarati et al. 2006; Syskova et al. 2007; Vodicka et al. 2001, 2004a, 2004b). The frequencies (mean ± SD) for chromatid-type aberrations (CTA) and chromosome-type aberrations (CSA) were 1.22 ± 1.21 and 1.15 ± 1.35, respectively. By analyzing modulating effects of genetic polymorphisms in GSTT1, GSTM1, and GSTP1 on CAs, CTAs, and CSAs (Table 1), we found no significant association between chromosomal damage and any of the studied polymorphisms. The results were further confirmed by logistic regression: for the GSTT1 null genotype, odds ratio (OR) = 1.35 [95% confidence interval (CI), 0.79–2.32; p = 0.27]; for the GSTM1 genotype, OR = 1.09 [95% CI, 0.74–1.62; p = 0.65]; and for a variant GSTP1 Val105Val genotype, OR = 0.83 [95% CI, 0.55–1.24; p = 0.36]. These data on CAs, CTAs, and CSAs (Table 1), distribution of analyzed genotypes and CA frequencies.

Table 1. Distribution of analyzed genotypes and CA frequencies.

| Genotype | CA | CTA | CSA |
|----------|----|-----|-----|
|          | Mean ± SD | χ² | p-Value | Mean ± SD | χ² | p-Value | Mean ± SD | χ² | p-Value |
| GSTT1 deletion | Plus | 23.26 ± 1.71 | 1.59 | 0.21 | 1.20 ± 1.21 | 0.88 | 0.35 | 1.11 ± 1.28 | 0.10 | 0.31 |
| Null | 7.4 | 2.50 ± 1.84 | 1.34 | 0.12 | 1.29 ± 1.47 | 0.32 | 0.56 | 1.29 ± 1.47 | 0.32 | 0.56 |
| GSTM1 deletion | Plus | 22.28 ± 1.77 | 0.43 | 0.51 | 1.25 ± 1.28 | 0.01 | 0.90 | 1.04 ± 1.32 | 1.11 | 0.29 |
| Null | 208 | 2.36 ± 1.70 | 1.20 | 1.17 | 1.18 ± 1.38 | 1.12 | 1.38 |
| GSTP1 Val105Val | Ile/Ile | 17.6 | 2.42 ± 1.91 | 2.25 | 0.33 | 1.31 | 1.38 | 0.36 | 0.84 | 1.12 | 1.34 | 1.27 | 0.53 |
| Val/Val | 7.36 | 2.58 ± 1.6 | 1.31 | 1.31 | 1.28 | 1.37 |

Data, pooled and recalculated from previously published data (Halasova et al. 2007; Musak et al. 2008; Naccarati et al. 2006; Syskova et al. 2007; Vodicka et al. 2001, 2004a, 2004b), were analyzed by the Kruskal-Wallis test.
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GST Polymorphisms: Bonassi et al. Respond
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We thank Vodicka et al. for drawing attention to a critical issue—the role of genetic polymorphisms in the induction of chromosomal damage.

First, we would like to clarify that our study (Rossi et al. 2009) was not designed to evaluate the influence of glutathione S-transferase (GST) polymorphisms on the frequency of chromosomal aberrations (CAs). Instead, we aimed to identify any possible susceptibility alleles that would increase the strength of the relationship between CAs and risk of cancer in subgroups of genetically variant individuals. The specific purpose of this case–control study explains the relative small size: We planned to obtain data on both CAs and genetic polymorphisms in the same individuals, with all subjects at ages appropriate to allow for an informative follow-up.

The interesting data reported by Vodicka et al. in their letter confirm the extreme heterogeneity of findings when the frequency of chromosomal damage is associated with polymorphisms in genes that influence several pathways. In fact, the lack of association in the whole database described by Vodicka et al. contrasts with the significant results reported in exposure-specific subsets, such as the highest frequency of CAs in GSTT1-null smokers working in the tire industry (Musak et al. 2008). This interpretation is also supported by the study of Iarmarcovai et al. (2008a), who reviewed 72 population studies reporting a significant effect of GSTM1, EPHX (microsomal epoxide hydrolase), and GSTT1 on micronucleus frequency. These authors also reported that the effect observed in specific pathways—such as ALDH2 in connection to chromosome damage due to alcohol consumption, BRCA1 and BRCA2 mutations in breast cancer patients, and MTHFR depending on folate levels—was much more pronounced.

A great deal of information about the role of genetic polymorphisms in the induction of chromosomal damage will stem from genome-wide association studies, when enough resources will be available in the field to explore the genetic determinants of chromosome damage using a whole genome scan. Nevertheless, classical association studies are still valid assuming that specific pathways are identified, possibly a priori, linking genotoxic exposure to polymorphisms of genes involved in the correspondent pathway.

We would also like to address future strategies for exploring the link between the frequency of chromosome damage and the risk of cancer (and other diseases). We fully agree with Vodicka et al.’s recommendation that possible modifying effects of lifestyle, occupational factors, and genetic polymorphisms be taken into account. However, after finding negative results concerning the role of occupational exposure to genotoxic agents and smoking habit (Bonassi et al. 2000), and polymorphisms of metabolic genes (Rossi et al. 2009), we believe that the remaining factor to be investigated is diet. Many studies have reported on the role of various food items and nutrients as modulators of chromosome stability (Fenech 2007); therefore, this parameter now has top priority, although reliable data are difficult to collect retrospectively.

The other recommendation from Vodicka et al. seems less promising, because measuring the frequency of chromosome damage in cancer patients (besides the classical discussion about whether the damage is a cause or a consequence of the disease) is possibly more a marker of progression than a predictive marker (Iarmarcovai et al. 2008b).

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Green Chemistry: Terminology and Principles
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We are grateful to Environmental Health Perspectives for implicitly embracing green chemistry as a field with profound connections to the environmental health sciences. We also commend the efforts of Wilson and Schwarzman (2009) to create greater transparency and accountability around chemicals of concern. We take issue, however, with their approach to key scientific concepts and terminology—specifically their effort to change the definition of “green chemistry.” Precision in terminology is paramount for science to function; all parties to the environmental health sciences. We take issue, however, with their approach to key scientific concepts and terminology—specifically their effort to change the definition of “green chemistry.”

In their review, Wilson and Schwarzman (2009) ignored the original and current definition of green chemistry, which for almost two decades has been recognized as a scientific discipline within the field of chemistry. Defined in the early 1990s by the U.S. Environmental Protection Agency (2009) as “the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances,” green chemistry is now guided by a set of 12 principles (Anastas and Warner 1998) that are used in both research and teaching in chemistry laboratories around the world.

Based on these principles, dozens of universities around the world teach green chemistry as a science. Seven graduate programs offer degrees in green chemistry. Two
established peer-reviewed scientific journals focus specifically on research in green chemistry. The impact factor of the journal *Green Chemistry* (published by the Royal Society of Chemistry) has increased from 2.5 to almost 5 over the past 5 years. More than 1,500 articles on green chemistry have been published in the scientific literature over the past 15 years.

Rather than embracing green chemistry’s widely used scientific definition, Wilson and Schwarzman (2009) instead conflate science and policy:

> The laws governing the chemical enterprise help define the incentives and disincentives that guide economic behavior in the market. … We use the term green chemistry in this context: as an analytical framework that encompasses both the science of safer chemistry and the laws and policies that will motivate its development and adoption by society.

This conflation brings with it two risks. First, it undermines clarity in scientific communication, something that is especially important as the fields of environmental health and green chemistry attempt to establish cross-disciplinary collaboration. Such collaborations are likely to prove vital for both fields. Second, it saddles the intellectual and scientific enterprise of green chemistry with policy and, potentially, political baggage, as considerations of chemical policies unfold in the political arena.

We are most certainly not arguing that the science of green chemistry should not inform chemical policies. Science and policy will be more effective, however, if political actors do not muddy accepted scientific terminology in service of a political/policy agenda, no matter how noble.

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**Green Chemistry: Wilson and Schwarzman Respond**

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We commend O’Brien, Myers, and Warner for their pioneering work identifying chemical threats to human and ecosystem health and for advancing the field of green chemistry. In our article (Wilson and Schwarzman 2009), we discussed advancing green chemistry as an objective of chemicals policy and described existing policy barriers that, if lifted, would spur the scientific and commercial development of green chemistry. O’Brien et al. recognize that the science of green chemistry has a role in informing chemicals policies. We would add that public policy that accurately reflects current science—and the needs of the chemicals market—is instrumental to the widespread adoption of green chemistry, but dispute that green chemistry is integral to chemicals policy itself.

Green chemistry’s potential is expanded when the science is embedded in a larger context. For example, in an introductory text on green chemistry, Lancaster (2002) stated that

> Green chemistry is not a new branch of science, but more a new philosophical approach that underpins all of chemistry and has technological, environmental and societal goals.

Lancaster (2002) pointed out that “over the last ten years, green chemistry has gradually become recognized as both a culture and a methodology for achieving sustainability,” and that the “12 principles of green chemistry help show how this can be achieved” (Lancaster 2002). This book addresses technical aspects of green chemistry while acknowledging the economic, legal, and knowledge barriers to advancing green chemistry.

> California has adopted a similarly expansive approach. The state’s 2-year-old Green Chemistry Initiative is rooted in the 12 principles of green chemistry, and it also embraces a range of tools to ensure the success of green chemistry in society. These include new regulatory strategies, economic incentives, technical assistance, research, and education, with the goal of “launch[ing] a new chemicals framework and a quantum shift in environmental protection” (Adams 2008). These are the same kinds of legislative and regulatory tools that California has used successfully to promote innovation in the energy sector, and which the empirical evidence has identified are the primary drivers for the adoption of cleaner technologies (Nameroff 2004). It is in this context that we see the broad potential of green chemistry.

> It is a credit to those who have established the field of green chemistry that multiple interests recognize its value in achieving environmental and economic sustainability, with benefits for worker health, resource conservation, environmental justice, public health, and global warming. As a result, green chemistry is reaching out from a core set of technical principles to inform broad societal goals.

As the field of green chemistry garners increasing attention in the scientific community and in society, it is worth recognizing that the 12 principles of green chemistry will be put to use in many contexts. We welcome the opportunity to join others in engaging with this promising field, with its relationship to the environmental health sciences and its role in effective public policy.

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*Authors’ joint statement: The five authors are currently working on a statement of shared views and goals.*

**Molybdenum Exposure and Semen Quality: How Robust Is the Evidence of an Effect?**

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Meeker et al. (2008) reported on associations between blood levels of nine different metals and four aspects of semen quality found in 219 volunteers attending two infertility clinics. Based on the title of their article, it seems that the authors claimed to have found evidence that molybdenum is a male reproductive toxicant. But how robust is the evidence? Meeker et al. (2008) had no primary prior hypotheses, and their study was essentially a “fishing expedition” involving many statistical tests of many possible associations. The authors (Meeker et al. 2008) did note that “some [findings] may
be chance findings because of the number of comparisons that were made.” A much stronger statement is appropriate, namely, that it is very likely that some of the findings were chance findings.

The overwhelming majority of study subjects (70%) had blood Mo levels below the study limit of detection of 1 µg/L. If a more sensitive assay method had been used [e.g., the method of Case et al. (2001)] with a detection limit of 0.06 µg/L, then a better grouping of subjects could have been used in the data analyses. We also believe the authors made an inappropriate analytical decision. In Table 3 of their article, Meeker et al. (2008) summarized analyses in which dose-dependent associations for each metal were considered in turn while adjusting for age and current smoking. The analysis of associations between Mo concentrations and semen motility was based on data from 147 subjects. The assumption appears to be that 72 (219 – 147) subjects had no useful information to provide on the Mo/sperm motility issue. It would have been more scientifically rigorous to allow the analysis of each semen parameter to make use of data from all study subjects, and not just for the sake of completeness. The current approach has introduced bias into the analysis, because different standards are applied to cases [subjects with the adverse health outcome of interest (e.g., poor sperm concentration)] and controls or referents (subjects without the adverse health outcome). To be concrete, in the analyses of sperm concentrations, cases were allowed to have poor sperm motility, whereas controls were not. The effect of this, in practice, is to load the controls with subjects on high incomes who have never smoked. A more standard analysis involving all study subjects in the analysis of each aspect of sperm quality should have been carried out. Such an analysis would likely have provided very different results to the subset analyses they reported.

In commenting on the linear regression analyses shown in their Table 4, Meeker et al. (2008) stated that the Mo groups “were associated with suggestive decreasing trends in sperm concentration and morphology.” However, examination of the table shows that neither of the regression coefficients was significantly different from zero (i.e., the blood Mo level had no significant influence on any of the semen quality parameters).

Additional studies would be needed to investigate possible effects in semen quality based on much larger population samples containing a wider range of blood Mo levels.

The authors are consultants for the International Molybdenum Association.

**Molybdenum Exposure and Semen Quality: Meeker et al. Respond**

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We appreciate the comments by Sorahan and Sullivan that serve to reiterate the limitations we discussed in our article reporting relationships between metal concentrations in blood and semen quality among 219 men recruited through two Michigan infertility clinics (Meeker et al. 2008). As highlighted in their letter, these limitations included the possibility of chance findings due to multiple comparisons and the sensitivity of the molybdenum assay used, among others. However, dismissing our analysis as a “fishing expedition” is not appropriate, given the animal evidence for adverse effects on male reproductive function in relation to Mo (Institute of Medicine 2001; Jeter and Davis 1954; Lyubimov et al. 2004; Pandey and Singh 2002; Thomas and Moss 1951; Van Niekerk and Van Niekerk 1989; Vyskocil and Viau 1999; Yamaguchi et al. 2007) and other metals. Because evidence exists for numerous metals to be either harmful or beneficial to male reproduction, and because metals may interact with one another synergistically or antagonistically, we conducted this exploratory study to provide the most comprehensive human data to date on multiple metals and semen quality. Because our study (Meeker et al. 2008) was the first to assess the relationship between Mo and semen quality in humans, we agree that our findings deserve further consideration and scrutiny through future research. An added impetus for further investigation comes from our recent follow-up analysis in which we reported a strong inverse association between Mo and circulating testosterone in these men, an association that was independent of semen quality (Meeker et al. 2009). Given that testosterone is not strongly predictive of semen quality parameters in this population (correlation coefficients < 0.15) or in other populations (Meeker et al. 2007)—but it does play a vital role in reproduction and other functions in males (e.g., metabolic disorders, bone health, endocrine-related cancers)—our findings suggest that multiple modes of Mo action on male reproduction may be possible and should be investigated.

We considered a number of approaches to model the association between metals in blood and semen quality parameters. In our logistic regression analysis (Meeker et al. 2008), we decided that including only men with all three semen quality parameters above reference values (i.e., “normal”) in the comparison group would be most appropriate, because the use of a heterogeneous comparison group that also includes men with compromised semen quality (i.e., at least one parameter below reference) may erroneously dilute the association between metals and semen quality parameters (Hauser et al. 2006). Sorahan and Sullivan’s assertion that the use of this more homogenous comparison group, which had normal levels of all three parameters, would somehow “overload” the comparison group with high-income nonsmokers in relation to the below-reference groups is unfounded since these factors were considered in the multivariable analyses. Finally, in response to Sorahan and Sullivan’s comment regarding our Table 4 (Meeker et al. 2008), in smaller to medium-sized studies it is common practice to use the term “suggestive” instead of “significant” in reporting associations with p-values between 0.05 and 0.15. There are many instances where relationships with a p-value just above 0.05 should not be completely disregarded, especially (as in this case) if they are consistent with results from other statistical analysis approaches or with previous (e.g., animal) research.

In conclusion, our findings (Meeker et al. 2008) may have large public health implications, but we agree that our study has several limitations. Our results need to be replicated in other large human studies, and potential biological mechanisms for the observed associations, and for metal–metal interactions, should be investigated in more detailed molecular epidemiologic, animal, and in vitro studies. Research is also needed on important sources, pathways, and routes that result in elevated Mo exposure, whether it stems from ingestion of naturally occurring or industrially related contamination of food and water, intentional ingestion of Mo-containing multivitamin/mineral supplements, potential multipathway exposure resulting from the use of Mo in flame
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retardants in building materials or consumer products, or a combination of these and other scenarios. Research comparing the utility of measuring Mo in blood, urine, and/or other matrices as biomarkers of Mo exposure over relevant time periods of interest is also needed.

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