Human Genome Epidemiology (HuGE) Review

A Meta-Analysis of the Association of N-Acetyltransferase 2 Gene (NAT2) Variants with Breast Cancer

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The N-acetyltransferase 2 gene (NAT2) product is an enzyme important in carcinogen metabolism via activation and detoxification pathways. Therefore, NAT2 variants may represent underlying susceptibility to breast cancer. Because a number of studies of the association of NAT2 with breast cancer have been published, the authors performed a meta-analysis. They extracted all relevant data to examine evidence for a main effect (i.e., the effect in a model that does not include any interactions) of NAT2 phenotype and genotype on breast cancer risk. They summarized the evidence for modification by smoking and meat intake, sources of exposure to aromatic and heterocyclic amines, respectively, which are metabolized by NAT2. The authors identified seven studies that measured NAT2 phenotype and 20 studies that deduced phenotype via genotyping. They found no evidence for heterogeneity (Cochran's Q statistic \( p = 0.74 \)) and no statistically significant increased risk from NAT2 acetylation (slow/rapid) for breast cancer (summary odds ratio \( = 1.02, 95\% \) confidence interval: 0.95, 1.08). These results suggest that there is no overall association between the NAT2 slow- or rapid-acetylation phenotype and breast cancer risk. However, some evidence suggests that smoking may modify this association.

acetyltransferases; breast neoplasms; epidemiology; genotype; meat; NAT2; polymorphism, genetic

Abbreviations: NAT2, N-acetyltransferase 2; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine.

Editor's note: This paper is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).

GENE

The N-acetyltransferase 2 gene (NAT2) is located on chromosome 8p21.3-23.1 and codes for a phase II xenobiotic metabolizing enzyme (1). The NAT2 enzyme plays an important role in the metabolism of both aromatic and heterocyclic amines, via N- and O-acetylation, which are pathways responsible for deactivation and activation, respectively (2).

GENE VARIANTS AND FREQUENCY

A previous NAT2 human genome epidemiology (HuGE) review detailed gene variants and their population frequencies (3). Briefly, NAT2 phenotypes are characterized as being slow, intermediate, or rapid acetylators, which refers to their ability to metabolize or activate xenobiotics.
DISEASE(S) OR OTHER OUTCOMES

Previous epidemiologic work implicates NAT2 variants in carcinogenesis, especially for bladder, colon, and breast cancer (3–5). This finding is biologically plausible owing to the ability of the NAT2 enzyme to N-acetylate or O-acetylate carcinogens to inert or noninert compounds capable of forming DNA adducts. Cigarette smoking and intake of well-done meat involve exposure to aryl and heterocyclic amines, substrates of NAT2; therefore, it is plausible that underlying NAT2 genotype may modify risk of cancer based on the ability to activate or detoxify heterocyclic and aromatic amines (6, 7).

OBJECTIVES OF THE CURRENT REVIEW

Since there have been a number of published reports on NAT2 and breast cancer, we conducted a meta-analysis to summarize the results of the effect on breast cancer of the NAT2 acetylation phenotype and NAT2 genotype and also to discuss evidence for an interaction for an interaction of NAT2 with exposure to smoking, meat intake, and meat cooking method.

METHODS

Study selection and inclusion criteria

We searched for relevant papers published before August 2006 by using the Human Genome Epidemiology Network (HuGENet) and the MEDLINE database (National Library of Medicine, Bethesda, Maryland). We searched MEDLINE by using the following terms: “arylamine N-acetyltransferase,” “acetyltransferases,” “NAT2,” “genetic polymorphism,” “restriction fragment polymorphism,” “single nucleotide polymorphism,” “breast cancer,” and “breast neoplasms.” We included all articles involving case-control or nested case-control studies that investigated NAT2 (determined by using phenotyping or genotyping methods) and breast cancer risk. We also searched the reference lists of the published studies found this way.

Data extraction

We extracted the following information from each manuscript: year of publication, country, number of cases and controls, matching, phenotyping technique, genotyping technique, alleles measured, allele and genotype frequencies, method for phenotype classification, phenotype frequencies, mean age of case and controls, menopausal status, covariates, and results by smoking or meat intake. We also extracted risk estimates for gene-environment interactions.

Classification of phenotype

Because of the inherent differences in methods for measuring NAT2 status—measuring phenotypes by using metabolic response to a particular compound or measuring alleles directly—we did not combine these studies for analysis. Respectively, these two types of studies are herein referred to as “phenotype” or “genotype” studies. Subsequently, we summarized phenotype frequencies (slow and rapid status). In the published genotype studies, individuals were classified as having a slow phenotype if homozygous for any of the slow-activity alleles and as having a rapid phenotype if homozygous or heterozygous for wild-type alleles. In some cases, we reclassified intermediate acetylators as rapid when the authors provided frequencies for an intermediate category (having one slow and one rapid allele) (6).

Statistical analysis

We performed a meta-analysis separately for phenotype and genotype studies. For each analysis, we investigated among-study heterogeneity by using Cochran’s Q statistic, and we examined fixed- and random-effect models based on the method of DerSimonian and Laird (8) in Number Cruncher Statistical software (9). We constructed a funnel plot to examine the influence of publication bias.

RESULTS

Phenotype studies

Seven studies were identified that analyzed differences in the proportion of cases and controls having a slow-acetylator phenotype (10–16). Table 1 summarizes these studies. Six were conducted in Europe, and the samples were primarily Caucasian. The sample size ranged from 79 to 515, totaling 1,330 women. Six studies measured acetylation phenotype via administration of arylamine drugs (four sulfamethazine, two dapsone); the other used isoniazid. We pooled the numbers across these studies and calculated the prevalence of the slow-acetylator phenotype to be 56 percent overall: 52 percent in cases and 58 percent in controls.

Three of the seven studies reported a higher prevalence of the rapid-acetylator phenotype in the breast cancer cases (10, 11, 15), but only two of the two studies were statistically significant (10, 15). For the meta-analysis, the test for heterogeneity was not statistically significant at p < 0.05 (Q statistic = 10.9, p = 0.09). Figure 1 shows the individual study odds as well as the summary odds ratio (0.77, 95 percent confidence interval: 0.61, 0.96) for the combined phenotype studies (where the reference group is rapid acetylators) using the fixed-effect model.

Genotype studies

Two duplication studies and two abstracts were identified that were later published as full manuscripts (17–20). These two particular full manuscripts were included in the meta-analysis. Another study, a case-cohort study (21), was brought to our attention by the reviewers of our paper. Excluding overlapping papers and including the case-cohort study, we identified 15 case-control studies, four nested case-control studies, and one case-cohort study, 20 in total (21–40). Table 2 summarizes these studies. Ten studies were population based, and eight were hospital based. Most studies were conducted among Caucasian populations. The only study that included a larger sample of African-American women was published by Millikan et al. (35). Three studies
TABLE 1. Description of phenotype-only studies included in a meta-analysis of the association of N-acetyltransferase 2 variants with breast cancer

<table>
<thead>
<tr>
<th>First author, year (reference no.)</th>
<th>Geographic region</th>
<th>Source/type of cases</th>
<th>Source/type of controls</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Method for phenotype determination</th>
<th>% with the slow phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulovskaya et al., 1978 (10)</td>
<td>Russia</td>
<td>Advanced breast cancer</td>
<td>Healthy, but some with age-associated cardiovascular disturbances</td>
<td>41</td>
<td>38</td>
<td>Sulfamethazine (not given)</td>
<td>31.7</td>
</tr>
<tr>
<td>Cartwright et al., 1984 (11)</td>
<td>United Kingdom</td>
<td>Invasive breast cancer</td>
<td>Not given</td>
<td>93</td>
<td>112</td>
<td>Dapsone (slow: MADDS/DDS*&lt;0.33)</td>
<td>45.2</td>
</tr>
<tr>
<td>Ladero et al., 1987 (13)</td>
<td>Spain</td>
<td>Histologically confirmed</td>
<td>Healthy or with disease not related to acetylator phenotype</td>
<td>81</td>
<td>75</td>
<td>Sulfamethazine (slow: ACMZ/SMZ*&lt;0.45 in plasma or &lt;0.77 in urine)</td>
<td>60.5</td>
</tr>
<tr>
<td>Philip et al., 1987 (14)</td>
<td>United Kingdom</td>
<td>Malignant breast disease</td>
<td>Cardiovascular disease and normal</td>
<td>181</td>
<td>337</td>
<td>Dapsone (slow: MADDS/DDS &lt;0.30)</td>
<td>54.7</td>
</tr>
<tr>
<td>Webster et al., 1989 (16)</td>
<td>United Kingdom</td>
<td>Histologically confirmed</td>
<td>68 healthy volunteers, 32 with breast abnormalities</td>
<td>100</td>
<td>100</td>
<td>Isoniazid (slow: ACINH/INH*&lt;1.5)</td>
<td>57.0</td>
</tr>
<tr>
<td>Sardas et al., 1990 (15)</td>
<td>Turkey</td>
<td>Advanced breast cancer</td>
<td>Healthy volunteers</td>
<td>28</td>
<td>51</td>
<td>Sulfamethazine (slow: not given)</td>
<td>39.3</td>
</tr>
<tr>
<td>Ilett et al., 1990 (12)</td>
<td>Australia</td>
<td>Surgical resection of primary breast carcinoma</td>
<td>No breast disease</td>
<td>45</td>
<td>48</td>
<td>Sulfamethazine (slow: ACMZ/SMZ &lt;0.6)</td>
<td>55.6</td>
</tr>
</tbody>
</table>

* MADDS/DDS, monoacetyldapsone/dapsone ratio; ACMZ/SMZ, N-acetylmethazine/methazine ratio; ACINH/INH, acetylisoniazid/isoniazid ratio.

FIGURE 1. Summary plot for phenotype studies (first author, date of publication (reference no.)) and combined estimate of odds ratio and 95% confidence interval. Reference group is rapid acetylators. Studies are sorted according to odds ratio. The sizes of the symbols are proportional to the study sizes.

were conducted among Asian populations (two in China and one in Korea) (29, 33, 39). Two recruited women based on menopausal status; the Iowa Women’s Health Study recruited postmenopausal women only (26), while Matheson et al. (34) included only premenopausal women. Of the 16,091 women included in the 20 studies, there were 7,479 cases and 8,612 controls.

Web table 1 shows the NAT2 alleles typed for each study as well as the frequency of slow- and rapid-acetylation phenotypes deduced from genotyping results by each author. (This information is described in the first of two supplementary tables; both are referred to as “Web table” in the text and are posted on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/reviews.htm) as well as on the Journal’s website (http://aje.oupjournals.org/).) In spite of the differences in nomenclature across the 20 genotype studies included in the meta-analysis, there were few actual differences in the particular alleles measured. In Caucasians, the majority of variation in slow-acetylator phenotype is due to three polymorphic sites (41, 42). Each study similarly measured the three alleles responsible for the majority of the slow-acetylator phenotype and classified women as having slow acetylators if they had two slow alleles. Therefore, carrying one copy of the *4 allele resulted in classification as a rapid acetylator. Seven studies measured additional rapid alleles (*12 or *13) (21, 26, 31–33, 38, 40), while six measured the *14 allele as an additional slow allele (23, 26, 27, 29, 35, 37). Intermediate acetylators were reclassified as rapid for the purpose of this review and, in each case the definition of intermediate was the same, as having only one rapid allele.
There is a confusion regarding differences in nomenclature for NAT2 alleles. An excellent review (6) as well as a website (www.louisville.edu/medschool/pharmacology/NAT.html) help to clarify the allele name with the specific nucleotide changes.

We reclassified intermediate acetylators as fast acetylators for the analysis in those studies in which the authors classified women into three categories (slow, intermediate, and rapid). Following this reclassification of intermediate phenotypes, we found that, overall, 54.4 percent of cases and 54.3 percent of controls were slow acetylators. From the 16 studies that included primarily Caucasian women and the Caucasian sample provided by Millikan et al. (35), we calculated prevalence of the slow genotype in Caucasians to be 56.9 percent: 57.0 percent in cases and 56.9 percent in controls. For the sample of African-American women, prevalence of the slow phenotype was 40 percent overall: 41 percent for cases and 40 percent for controls (35). In the three Asian studies, the pooled prevalence of the slow phenotype was 20 percent: 21 percent in cases and 18 percent in controls.

Associations

Web table 2 shows that three of the 20 studies reported a significant association between NAT2 acetylation and breast cancer. Alberg et al. (23) showed that rapid-acetylation status increased risk both in the whole sample and among postmenopausal women. Conversely, Sillanpaa et al. (37) in the Finnish sample and Huang et al. (29) in the Taiwanese population reported an increased risk for slow acetylators, which may also be highest for postmenopausal women. For the meta-analysis of the genotype studies, we found no evidence for among-study heterogeneity ($Q$ statistic = 14.58, $p = 0.75$). Figure 2 shows the individual odds ratios, as well as the summary odds ratio (1.02) and 95 percent confidence interval (0.95, 1.08) for the main effect of NAT2 acetylator genotype obtained by using a fixed-effect model (8).

Figure 3 is a funnel plot showing the association of NAT2 with breast cancer by study type (phenotype or genotype) and study size. There does not appear to be an obvious publication bias toward positive or negative findings among the genotype studies. However, in the phenotype studies, there may be a publication bias toward manuscripts showing risk associated with rapid phenotypes because the plot is asymmetrical.

Smoking

In terms of a main effect of smoking, 15 of the 20 genotype studies analyzed smoking and breast cancer risk by using a variety of exposure variables: smoking status (active, former, passive smoking), cumulative exposure, duration, and intensity via number of cigarettes smoked per day (which may have been measured as many as 20 years prior to diagnosis). Of these 15 studies, nine found no significant association between smoking and breast cancer risk. Hunter et al. (30) reported increased risk for women who smoked 15 or more cigarettes per day (10 years prior to diagnosis) and women with 20–30 pack-years of exposure, a finding replicated by Millikan et al. (35), who reported increased risk of breast cancer with more than a 20-year duration of active smoking. Morabia et al. (36) reported increased risk for current and former smokers. Egan et al. (28) found increased risk for ever smokers and increasing pack-years; however, the association with increasing pack-years was significant for postmenopausal women only. Lissowska et al. (40) reported increased risk for women less than age 45 years in terms of smoking status, increasing number of cigarettes per day and duration, and age at which smoking started. None of the studies showed a decreased risk of breast cancer with active or passive smoking.

Interactions

Smoking. Of the 15 genotype studies that measured smoking, 13 investigated the hypothesized interaction of smoking and NAT2 acetylator genotype. Some studies further stratified by menopausal status.

Among the studies that examined the $NAT2 \times$ smoking interaction according to menopausal status, Ambrosone et al. (24) was the first to show increased risk of breast cancer for slow-acetylator, postmenopausal women who were current or former smokers (using cigarettes/day 2 years ago, cigarettes/day 20 years ago, and lifetime pack-years). These findings were later replicated by Egan et al. (28) (pack-years) and Alberg et al. (23) (active smoking), who reported the strongest association among postmenopausal women. Sillanpaa et al. (37), van der Hel et al. (38), and Matheson et al. (34) reported significant increased risk for smokers who were slow acetylators.

Krajnovic et al. (32), Delfino et al. (27), Hunter et al. (30), and Millikan et al. (35) showed no significant evidence for modification of $NAT2$ and breast cancer by smoking. Krajnovic et al. reported a nonsignificant increase in risk among rapid acetylators who were current or former smokers, which became significant in the case-only analysis. Delfino et al. reported no trend in risk for rapid or slow acetylators, while Hunter et al.’s weak, nonsignificant trend was among slow acetylators. Millikan et al. reported an increased, yet nonsignificant trend in risk for duration of smoking among postmenopausal rapid acetylators. Interestingly, Chang-Claude et al. (25) reported increased risk of breast cancer in rapid acetylators exposed to passive smoke, a finding later replicated by Morabia et al. (36).

Two of the studies conducted a case-only analysis. Results from Ambrosone et al. (24) suggest that, among cases, the odds of being a slow acetylator were greatest for women who smoked more than 20 cigarettes per day (20 years ago), smoked for more than 20 pack-years, or initiated smoking at an earlier age ($\leq$ 16 years). Conversely, Krajnovic et al. (32) reported increased odds for being a rapid acetylator for ever smokers.

Meat intake. There is much published literature on the association of breast cancer with red meat intake and meat doneness (43). Five of the studies included in this review investigated potential modification of the association of $NAT2$ with breast cancer according to charred or well-done meat intake. The only study that found evidence for
interaction between meat doneness and NAT2 status was that of Deitz et al. (26), who reported a significant increasing trend in risk among rapid acetylators with increasing meat doneness score.

Ambrosone et al. (44) reported no significant association between meat consumption and breast cancer, nor did they find evidence of a significant interaction between meat intake and NAT2 acetylation status. Similarly, Gertig et al. (45) found no association of red meat intake or meat cooking method with breast cancer risk in the Nurses’ Health Study, and no significant interaction with NAT2. Delfino et al. (46) also found no evidence of increased risk for intake of well-cooked meat nor an interaction with NAT2. The last of the studies, by Krajinovic et al. (32), did not report a significant elevated risk for rapid acetylators who consumed well-done meat. In each of these studies, the trends in risk according to increasing meat intake or charred meat intake were not strong enough to suggest whether NAT2 slow or rapid acetylators are at increased or decreased risk.

DISCUSSION

Main findings

The results of this meta-analysis show no evidence for an overall effect of NAT2 acetylation capacity on the risk of breast cancer. There is suggestive evidence that acetylation may underlie a susceptibility to breast cancer if there is also exposure to tobacco smoke, but there is little evidence for effect modification by intake of well-done meat. Future studies evaluating gene variants in entire pathways may enable better evaluation of susceptibility to breast cancer in the presence of other factors.

Table continues
There are a number of challenges in summarizing studies of NAT2 variants; there are not only differences in single nucleotide polymorphism selection and technology available at the time of the study but also a reliance on phenotypic characterization of NAT2 variants. Any misclassification of individuals as slow, intermediate, or rapid acetylators would more likely result in bias toward the null and therefore may partly explain the lack of an overall significant association between NAT2 acetylation status and breast cancer.

Following construction of a funnel plot (figure 3) of both phenotype and genotype studies, we conclude that there is some degree of publication bias in the phenotype studies. These studies were more likely to be published if they found a protective effect for slow acetylators. The distribution of odds ratio estimates in the genotype studies suggests that, in these publications, bias is less likely to have occurred.

Our original goal was to also conduct a quantitative review of the potential factors implicated in modifying the association of NAT2 with breast cancer, specifically smoking and meat intake. However, once we examined the studies and discovered the many different exposure assessments used to classify individuals, we concluded that the difficulty associated with summarizing the effect of different exposure variables would be better overcome by using a pooled design in which primary data are obtained from authors of published manuscripts. At the same time, we found that a pooled analysis, in which the exposure measurements can be better summarized and a more comprehensive analysis performed, is already under way.

**Biology**

Approximately 26 NAT2 variants are known to exist in humans, some silent and some functional variants responsible for changes in enzymatic activity. An excellent review...
and explanation of genotype-phenotype correlation was published by Hein et al. (6). This paper helps to clarify the confusion regarding NAT2 allelic nomenclature as well as the functional significance and classification of the different alleles.

For the phenotype studies, our meta-analysis suggests evidence of decreased breast cancer risk for women with a slow phenotype (odds ratio = 0.77, 95 percent confidence interval: 0.62, 0.96) or, conversely, increased breast cancer risk for women with a rapid phenotype (odds ratio = 1.29, 95 percent confidence interval: 1.04, 1.62) when slow is the reference group. The published reports also suggest increased risk for rapid acetylators; however, the majority of these studies were unable to report a significant association. Evans (47) combined the results of Bulovskaya et al. (10) and Cartwright (11) and found an odds ratio of 2.05, suggesting an increased risk for carriers of the rapid allele. These studies were of limited size, however, which may have increased sampling error. In addition, misclassification may also be important because different xenobiotics and methods were used to assess NAT2 activity. The NAT2 phenotype may be influenced by disease status; therefore, the results of the genotype studies may be more reliable because genotype is not affected by disease status.

The genotype studies purport a modifying effect of smoking in the association of NAT2 with breast cancer, especially in postmenopausal women. Postmenopausal women are more likely to have a greater duration of smoking simply because of their age, which may explain these findings. These studies therefore suggest a role for NAT2 in breast cancer carcinogenesis, especially for women who also smoke. This hypothesis can be supported by evidence from Pfau et al. (48), who showed that slow acetylators have a higher quantity of DNA adducts than rapid acetylators. A recent review and meta-analysis of the potential modifying effect of smoking suggests that, among slow acetylators, smoking may increase breast cancer risk, an effect that is strongest among postmenopausal women (odds ratio = 2.4, 95 percent confidence interval: 1.7, 3.3) (49), which agrees with our assessment of the studies included in our review.

The increased risk of breast cancer for women exposed to passive smoke who are also rapid acetylators is very interesting (25, 36) in that passive smoke contains 2-amino-acarboline, a heterocyclic amine (50). If exposed to passive or sidestream smoke, rapid acetylators are at increased risk since they are more likely to O-acetylate the N-hydroxy heterocyclic amines that have been processed in the liver by CYP1A2 and subsequently form compounds capable of creating DNA adducts (50). Further studies are needed to confirm this association.

Biologically, slow acetylators who have lower concentrations of active NAT2 enzyme and are exposed to aromatic amines are more likely to undergo metabolism via the hydroxylation pathway. This process may then lead to electrophilic intermediates capable of binding DNA and initiating carcinogenesis, as compared with the process in rapid acetylators, whose aromatic amine exposure is metabolized via N-acetylation to become an inactive metabolite (51). Firozi et al. (52) reported a higher frequency of smoking-related

FIGURE 2. Forest plot of genotype studies (first author, date of publication (reference no.)) and combined odds ratio and 95% confidence interval. Reference group is rapid acetylators: those with at least one rapid N-acetyltransferase 2 allele. Studies are sorted according to odds ratio. The sizes of the symbols are proportional to the study sizes.

FIGURE 3. Funnel plot of the association of the N-acetyltransferase 2 (NAT2) genotype with breast cancer, by study size. Odds ratios for the main effect of NAT2 are shown. Reference group is rapid acetylators.
adducts in breast cancer cases with the slow-acetylation phenotype, which suggests that the hypothesized increased risk of breast cancer and smoking is especially important for slow acetylators.

The varying action of NAT2 under different exposure scenarios may partially explain the null findings for the main effect of NAT2 and breast cancer. While smoking involves exposure to both aromatic amines and heterocyclic amines, N- and O-acetylation may be taking place. Thus, the competing or heterogeneous nature of the pathway depending on exposure may attenuate the association. In spite of this heterogeneity, many of the studies found evidence of increased risk for slow-acetylating women who also smoke.

Another exposure that has been investigated as a potential modifier of the NAT2 and breast cancer association is meat intake. Meat intake, especially intake of charred meat, is associated with exposure to heterocyclic amines because high meat intake results in increased exposure to 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP) (53). PhIP is the most abundant heterocyclic amine in cooked meat (53). Women with the rapid-acetylation phenotype and high intake of well-done or charred meat may especially be at increased breast cancer risk, since O-acetylation by NAT2 predominates, resulting in reactive metabolites capable of forming DNA adducts and mutations. Zhu et al. (54) detected PhIP-DNA adducts in breast tissue and reported that rapid acetylators, compared with slow acetylators, had a higher level of PhIP-DNA adducts, conferring increased risk of breast cancer in rapid acetylators who have high exposure to PhIP.

Regarding NAT2, the molecular explanation for why particular exposures more readily result in N- or O-acetylation may be an important cause of the lack of significant main effect of the NAT2 genotype and breast cancer risk. The results of the meta-analysis of the genotype studies are therefore not unexpected. Aryl and heterocyclic amines differ in how they are metabolized, namely, the propensity toward N- and O-acetylation pathways for these respective exposures. Without specific knowledge of which pathway may predominate and whether other genes are involved, it is difficult to discern epidemiologically the exact estimate of risk conferred via one particular gene. Future studies should also measure and analyze the interaction of NAT2 and other genes, such as CYP1A2, in the carcinogen metabolism pathway since knowledge of underlying variation in both genes may more fully characterize an individual’s carcinogen metabolism phenotype (55).

Future studies that investigate smoking and meat intake should attempt to collect and use standardized exposure measures. Doing so would greatly help summarize the results of published studies.

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