Polymorphisms in GSTT1, GSTZ1, and CYP2E1, Disinfection By-products, and Risk of Bladder Cancer in Spain

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BACKGROUND: Bladder cancer has been linked with long-term exposure to disinfection by-products (DBPs) in drinking water.

OBJECTIVES: In this study we investigated the combined influence of DBP exposure and polymorphisms in glutathione S-transferase (GSTT1, GSTZ1) and cytochrome P450 (CYP2E1) genes in the metabolic pathways of selected by-products on bladder cancer in a hospital-based case–control study in Spain.

METHODS: Average exposures to trihalomethanes (THMs); a surrogate for DBPs; from 15 years of age were estimated for each subject based on residential history and information on municipal water sources among 680 cases and 714 controls. We estimated effects of THMs and GSTT1, GSTZ1, and CYP2E1 polymorphisms on bladder cancer using adjusted logistic regression models with and without interaction terms.

RESULTS: THM exposure was positively associated with bladder cancer: adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were 1.2 (0.8–1.8), 1.8 (1.1–2.9), and 1.8 (0.9–3.5) for THM quartiles 2, 3, and 4, respectively, relative to quartile 1. Associations between THMs and bladder cancer were stronger among subjects who were polymorphic in human populations, with the GSTT1 deletion resulting in lack of enzyme activity, and with several nonsynonymous single-nucleotide polymorphisms (SNPs) in GSTZ1 and CYP2E1 resulting in modified enzymatic activity (Blackburn et al. 2001; Bolt et al. 2003). We hypothesized that one or more of these functional polymorphisms could influence bladder cancer risk posed by DBPs, and we investigated this in a large case–control study in Spain, where in a previous study (Villanueva et al. 2007) we observed elevated risk of bladder cancer after long-term exposure to DBPs.

MATERIALS AND METHODS: We conducted a hospital-based case–control study in 18 hospitals located in five areas of Spain [Asturias, Barcelona metropolitan area, Valles/Bages (including the municipalities of Manresa and Sabadell), Alicante, and Tenerife (Appendix 1)]. Eligible cases were 21–80 years of age, newly diagnosed with histologically confirmed urothelial carcinoma of the bladder between 1998 and 2001, and living in the catchment geographic area of

Chlorine is a cost-effective drinking water disinfectant that has been used since the early twentieth century to control a panoply of waterborne infectious diseases. By-products of the interaction of chlorine with organic precursors in water were first noted in 1974, with the discovery of trihalomethanes (THMs) in disinfected water (Bellar and Lichtenberg 1974; Rook 1974). Since then, hundreds of halogenated chemical species in the disinfection by-product (DBP) mixture have been detected, including both brominated and chlorinated compounds (Richardson 2003). THMs and haloacetic acids (HAAs) are the chemical groups at highest concentration in most by-product mixtures. Toxicological and epidemiologic studies of DBPs provide evidence of elevated risk of cancer and adverse birth outcomes (Cantor et al. 2006; Grellier et al. 2010; Nieuwenhuijsen et al. 2009). In particular, human bladder cancer has been consistently linked with long-term exposure (Cantor et al. 1998; King and Marrett 1996; McGeehin et al. 1993; Villanueva et al. 2004).

These observations are supported by evidence of mutagenicity of the mixture and carcinogenicity of some constituents (Komulainen et al. 1998; King and Marrett 1996; Pegram et al. 1997), and bladder cancer has not been studied in this regard.

At least three enzymes in the metabolic pathways of DBP components are candidates for examination. Glutathione S-transferase (GST) theta-1 (GSTT1) activates brominated THMs to mutagens in a transgenic strain of Salmonella (DeMarini et al. 1997; Pegram et al. 1997). GST zeta-1 (GSTZ1) catalyzes the oxygenation of dichloro- and other α-haloacids, some of which are animal carcinogens (DeAngelo et al. 1999; Melnick et al. 2007; Tong et al. 1998). Cytochrome P450 2E1 (CYP2E1) metabolizes a wide variety of aliphatic hydrocarbons, solvents, and industrial monomers (Guengerich et al. 1991) and is responsible for the primary oxidation of THMs. Genes that code for these enzymes are polymorphic in human populations, with the GSTT1 deletion resulting in lack of enzyme activity, and with several nonsynonymous single-nucleotide polymorphisms (SNPs) in GSTZ1 and CYP2E1 resulting in modified enzymatic activity (Blackburn et al. 2001; Bolt et al. 2003). We hypothesized that one or more of these functional polymorphisms could influence bladder cancer risk posed by DBPs, and we investigated this in a large case–control study in Spain, where in a previous study (Villanueva et al. 2007) we observed elevated risk of bladder cancer after long-term exposure to DBPs.

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participating hospitals. Cases were identified from the registers of urological services augmented by regular and frequent evaluations of hospital discharge records, pathology records, and local cancer registries. A panel of expert pathologists confirmed diagnoses and ensured uniformity of classification criteria, based on the 1998 World Health Organization/International Society of Urological Pathology system (Epstein et al. 1998).

Controls were selected from patients admitted to participating hospitals with conditions thought to be unrelated to the major risk factors of bladder cancer, such as tobacco use. Diagnostic categories for controls were as follows: 37% hernias, 11% other abdominal surgery, 23% fractures, 7% other orthopedic problems, 12% hydrocele, 4% circulatory disorders, 2% dermatological disorders, 1% ophthalmological disorders, and 3% other diseases. Controls were individually matched to cases by age at interview within 5-year strata, sex, ethnic origin, and hospital catchment area, a well-defined area corresponding to the specific health services region covered by each hospital. Written informed consent was obtained from each subject before the study. The study was approved by the review board of each participating institution and in accord with an assurance filed with and approved by the U.S. Department of Health and Human Services.

Individual data. After obtaining informed consent, trained interviewers administered a computer-assisted personal interview (CAPI) to participants during their hospital stay. Interview items included sociodemographic characteristics; smoking habits; occupational, residential, and medical histories; and familial history of cancer. We identified 1,457 eligible cases and 1,465 eligible controls. Of these, 84% of cases (n=1,219) and 87% of controls (n=1,271) participated. Subjects who refused to answer the CAPI were administered a reduced interview of critical items (21% of cases and 19% of controls). Questionnaire information on water-related exposures used in this analysis included residential history from birth (all residences of at least 1 year, drinking water source at each residence (municipal/bottled/private well/other)) and swimming pool use as an adult. These data were collected from all participants, including those who responded to the critical items questionnaire.

Exposure data. Using a structured questionnaire, we collected historic water quality data from approximately 200 local authorities and 150 water companies in the study areas. For 123 study municipalities, covering 78.5% of the total study exposure-years, we obtained annual average THM levels. In addition, one of us (C.M.V.) measured levels of the four THMs [chloroform, bromodichloromethane (BDCM), dibromochloromethane, and bromofom] in 113 tap water samples from the studied geographic areas between September and December 1999.

Average THM levels in recent years were extrapolated back to approximately 1920. Historical THM levels were estimated by municipality under the assumption that past THM levels were similar to current concentrations when the water source had not changed. When the water source had changed, we calculated the average THM level using the proportion of surface water during the relevant time period. We assumed that the THM level before the start of chlorination was zero. The exposure assessment was described previously (Villanueva et al. 2006, 2007).

Lifetime individual exposure indices. We merged individual and municipal databases with individual residential information by year and municipality, and obtained individual yearly-average THM levels, water source, and chlorination status for each study subject. We used as an exposure metric the average THM level of the water source serving participating residences in the period between age 15 years and the interview, as described previously in the analysis of the main effects of DBP exposure (Villanueva et al. 2007). As in that study, we restricted our analysis to subjects with a household THM estimate for at least 70% of the years in this exposure window.

Genotyping. DNA for genotype assays was extracted from leukocytes or mouthwash samples as described previously (Garcia-Closas et al. 2005). Genotype assays for polymorphisms in GSTT1, GSTM1, and N-acetyltransferase 2 (NAT2) were conducted at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics (National Cancer Institute). The SNPs of NAT2 and assignments of rapid/intermediate/slow acetylator types, as well as the GSTM1 genotypes, have been described in detail elsewhere (Garcia-Closas et al. 2005). GSTT1 genotypes were defined as null (−/−) if a deletion was found in both copies of the gene and present if one (+/−) or none (+/+). The copies had a deletion. We used the TaqMan assay (Applied Biosystems, Foster City, CA, USA) for SNPs in NAT2, two of the three SNPs in GSTZ1 (rs6326; rs6326) and GSTM1 (rs7978, rs7978, and deletions in GSTT1 and GSTM1). The methods for each specific assay are available from the National Cancer Institute (2010). A third SNP in GSTZ1 (T827C), three and three SNPs in CYP2E1 (IVST-118C>G rs2070567, −1054G>T rs2031920, and −1514G>T rs8192760) were determined in a GoldenGate assay (Illumina, San Diego, CA, USA) (Garcia-Closas et al. 2007). All genotypes studied were in Hardy-Weinberg equilibrium in the control population. Duplicate quality control samples (n=93 pairs) showed ≥99% agreement for all assays.

Statistical analysis. We considered results to be statistically significant if the alpha level was ≤0.05. Subjects were grouped by quartile of the time-weighted average THM level since age 15 years. We used unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). ORs were adjusted for age (continuous), sex, smoking status (never/former/current), size of the municipality of longest residence until 18 years of age (reported by the participant as metropolitan, city, small city, village, or farm), education (three strata: less than primary school, less than high school, and high school or more), geographic area (six strata: Barcelona, Sabadell, Manresa, Alicante, Tenerife, and Asturias), and overall quality of interview (reported by interviewer as unsatisfactory, questionable, reliable, or high quality). Analyses using more detailed smoking information (total duration, average daily number of cigarettes, tobacco type, pack-years) gave similar results and are not reported. Subjects were defined as “former” smokers if they had quit at least 1 year before the interview. Missing data for covariates were coded to a separate category for each variable included in the models. This applied to a small proportion of respondents for each covariate. For example, <1% of respondents were missing data on smoking status (Samanic et al. 2006). Linear p-trends were calculated using a likelihood ratio test comparing the model with and without the THM exposure variable modeled as a continuous variable with each quartile coded according to its median value.

Interactions between genotypes and exposure to DBPs, as estimated by average THM levels serving the household between 15 years of age and diagnosis (cases) or interview (controls), were also tested using the likelihood-ratio test to allow estimation of parameters under the assumption of genotype–DBP (THMs) independence in the source population. Tests for multiplicative interaction were used to assess whether the genotype ORs within categories of DBP exposure or, equivalently, DBP ORs within genotype categories differed significantly from each other. Haplotype frequencies for GSTZ1 and CYP2E1 were estimated using HaploStats (version 1.2.1; http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm) using the program language R (http://www.r-project.org/).

Results. Of the 1,219 cases and 1,271 controls interviewed, 1,188 (97%) cases and 1,173 (92%) controls provided a blood or buccal cell sample for DNA extraction. After excluding cases and controls with low amounts of DNA, nonwhite individuals (to limit heterogeneity), and DNA quality control difficulties, the study population with adequate genetic material available for analysis numbered 1,150 cases and
interaction between average THM exposure and polymorphic forms of three genes:

| Measure | n (cases/controls) | OR (95% CI) | p-Value |
|---------|-------------------|-------------|---------|
| Average THM level at home (μg/L, age 15 to index age) | | | |
| ≤ 8.0 | 156/175 | 1.0 | |
| > 8.0–26.0 | 153/174 | 1.2 (0.8–1.9) | 0.27 |
| > 26.0–49.0 | 197/179 | 1.8 (1.1–2.9) | 0.02 |
| > 49.0 | 174/196 | 1.8 (0.9–3.5) | 0.13 |

**Table 1. ORs (95% CIs) for bladder cancer for quartile levels of average long-term level of household THMs and for selected SNPs.**

For all associations between THM exposure and polymorphic forms of the genes examined, we found no evidence of interaction (p<interaction > 0.052, data not shown).

### Table 2. Interaction between average THM exposure and polymorphic forms of three genes: GSTTI, GSTZ1, and CYP2E1.

| Gene, average THM (μg/L) | n (cases/controls) | OR (95% CI) | p-Value |
|-------------------------|-------------------|-------------|---------|
| GSTTI Null | 34/34 | 1.0 (reference) | 1.0 (reference) |
| > 8.0–26.0 | 36/37 | 1.2 (0.5–2.5) | 0.27 |
| > 26.0–49.0 | 37/41 | 1.2 (0.5–2.5) | 0.24 |
| > 49.0 | 29/48 | 1.0 (0.4–2.5) | 0.24 |

**Table 2. Interaction between average THM exposure and polymorphic forms of three genes: GSTTI, GSTZ1, and CYP2E1.**

*Numbers of cases or controls per measure may not equal the total number of cases (680) and controls (714) because of missing genotype data. ORs (95% CIs) from logistic regression adjusted for age (continuous), sex, smoking status (never/former/current), size of the municipality of longest residence until 18 years of age, education (three strata), geographic area (six strata), and overall quality of interview.*

### References

1. Garcia-Closas, M., et al. (2005). Bladder cancer, DBPs, and genetic polymorphisms. *Environ Health Perspect.* November 2010. 1547.
subjects with both the variant and common forms of CYP2E1 rs2031920 (Table 3). Among subjects with both GSTT1 present and GSTZ1 CC/T/T genotype, OR increased monotonically to 5.9 (95% CI, 1.8–19.0) in the highest quartile of THMs ($P_{trend} = 0.0012$). In contrast, among subjects with both GSTT1 null and GSTZ1 CC genotypes, we found no increase in relative risk with increasing THM level ($P_{interaction} = 0.0052$). These analyses excluded subjects with both low-risk variants of GSTZ1 and high-risk variants of GSTT1, or vice versa. When we restricted this analysis to subjects with the common form (CC) of CYP2E1 rs2031920, among those with both GSTT1 present and GSTZ1 CC/T/T genotypes, the OR increased monotonically to 9.3 (2.5–34.0) in the highest quartile of THM exposure ($\geq 49.0 \mu g/L$), relative to participants with these genotypes in the lowest quartile ($\leq 8.0 \mu g/L$). $P_{trend} = 0.010$; see Supplemental Material, Table 1 (doi:10.1289/ehp.1002206).

We calculated the main effects for the respective SNPs within increasing quartile strata of long-term average THMs (Table 4) where we found increasing relative risks for bladder cancer for GSTT1 present, GSTZ1 rs1046428 C/T/T, and CYP2E1 rs2031920 CC. Among subjects with exposure to THMs in the highest quartile, the ORs were elevated and the 95% CIs excluded 1.0 for GSTT1 present (vs. GSTT1 null), GSTZ1 (C/T/T vs. CC), and CYP2E1 CC (vs. C/T/T), whereas ORs for these polymorphisms were close to 1.0 among subjects in the lowest THM exposure quartile. The associations with bladder cancer risk for NAT2 slow versus rapid/intermediate acetylator and GSTM1 null versus present within each THM stratum were variable and consistent with the overall elevated main effects for these genes.

In earlier analyses of these data, we found a significant association with ever swimming in pools [OR = 1.62 (95% CI, 1.20–2.19)] but no association with increasing hours of lifetime pool use (Villanueva et al. 2007). There was no significant difference in risk for ever swimming in pools with any of the genetic polymorphisms under evaluation here. The weak statistical interactions observed with GSTT1 present (vs. GSTT1 null) and with GSTZ1 CC/T/T (vs. CC) were in a direction contrary to expectation, given what we observed for interactions with THMs [see Supplemental Material, Table 2A,B (doi:10.1289/ehp.1002206)].

**Discussion**

DBPs were previously found to be a bladder cancer risk factor in this case–control study in Spain, as well as in other settings (Villanueva et al. 2004, 2007). In the present study we found significant differences in the dose–response relation of bladder cancer risk with increasing average long-term exposure to DBPs (as represented by THMs) among subjects with differing genotypes in each of three candidate genes. GSTT1 and GSTZ1 code for enzymes in the respective biotransformation pathways of two groups of DBPs, the brominated THMs and the α-halocarbons. CYP2E1 oxidizes THMs and likely many other compounds in the DBP mixture. Overall, without considering DBP effects or interaction, we found a weak, nonsignificant overall association between polymorphisms in each of these genes and bladder cancer risk. Associations between increasing quartiles of THMs and bladder cancer were stronger among subjects with GSTT1 +/+ or +/− versus −/−, GSTZ1 rs1046428 CT or TT versus CC, and CYP2E1 rs2031920 CC versus CT (no subjects had TT), with statistically significant interactions for each of the respective gene variants. Among the 195 cases and 192 controls with GSTT1 present and GSTZ1 rs1046428 CT/TT, ORs (95% CIs) for quartiles 2, 3, and 4 of long-term average THMs were 1.5 (0.7–3.5), 3.4 (1.4–8.2), and 5.9 (1.8–19.0), respectively, relative to quartile 1. We also found that main effect estimates for bladder cancer in association with these polymorphisms varied by THM level, with significant associations for all three high-risk genotypes among subjects within the highest quartile of long-term THMs (Table 4). The supermultiplicative interactions between residential water THM level and polymorphisms in GSTT1, GSTZ1, and CYP2E1 are consistent with the hypothesis that these genes influence the metabolism of carcinogens in the DBP mixture in drinking water.

GSTZ1, conserved over a long evolutionary period, plays a key role in the catalysis of phenylalanine and tyrosine. In addition, GSTZ1 transforms several xenobiotic α-haloacid substrates. The HAAs dichloroacetic acid, bromochloroacetic acid, and dibromoacetic acid are transformed to glyoxylic acid, and 2,2-dichloropropionic acid is metabolized to pyruvic acid (Board and Anders 2005). In rodents, dichloroacetic acid causes liver cancer and dibromoacetic acid is a multistatic carcinogen (DeAngelo et al. 1999; Melnick et al. 2007). Among GSTZ1-depleted rats, total body clearance of dihaloacetic acids was 3–10 times lower than in rats with normal levels of GSTzeta (Saghir and Schultz 2005). Several SNPs in the GSTZ1 gene are known (Blackburn et al. 2001). Notably, the rs1046428 T allele has been observed to have low enzymatic activity for transformation of dichloroacetic acid compared with the C allele (Blackburn et al. 2001), consistent with our finding of elevated risk for individuals with this genotype. Many other α-halocarbons in the DBP mixture that have not been tested for carcinogenicity may also serve as substrates for GSTZ1 and thereby participate in the transformation we observed. Haplotype analysis for GSTZ1 did not reveal notable elevated or lowered risk of bladder cancer.

A common polymorphic variant of GSTT1 is the null form of the allele, which is associated with lack of enzymatic activity. About 20% of Caucasians are homozygous null for this gene (Raimondi et al. 2006). In our control population, 22.5% were homozygous null. Brominated THMs are mutagenic and carcinogenic and are among the

**Table 3. ORs (95% CIs) for the combined effects of polymorphic forms of GSTT1 and GSTZ1.a**

| Average THM (µg/L) | GSTT1 null and GSTZ1 CC | GSTT1 present and GSTZ1 CC/T/T | $P_{trend}$ | $P_{interaction}$ |
|------------------|------------------------|-------------------------------|-------------|-----------------|
| $\leq 8.0$        | 19/17                  | 0.9 (0.5–1.5)                 | 0.8 (0.5–1.5) | 1.3 (0.8–2.0) | 0.0012           | 0.0052           |
| > 8.0–26.0       | 25/24                  | 0.9 (0.5–1.6)                 | 1.0 (0.6–1.6) | 1.2 (0.7–2.0) | 1.0 (0.6–1.6) | 0.0012           | 0.0052           |
| 26.0–49.0        | 21/29                  | 1.0 (0.6–1.6)                 | 1.5 (0.9–2.5) | 1.6 (1.0–2.5) | 1.0 (0.6–1.6) | 0.0012           | 0.0052           |
| > 49.0           | 17/16                  | 1.0 (0.6–1.6)                 | 1.5 (0.9–2.5) | 1.6 (1.0–2.5) | 1.0 (0.6–1.6) | 0.0012           | 0.0052           |

aOR (95% CI) from logistic regression adjusted for age (continuous), sex, smoking status (never/former/current), size of the municipality of longest residence until 18 years of age (three strata), geographic area (six strata), and overall quality of interview.
most prevalent DBPs in chlorinated drinking water. Pegram et al. (1997) and DeMartini et al. (1997) demonstrated that in Salmonella transfectet with plant GSTT1+ , brominated THMs are activated to mutagens, and they identified the specific mutagenic transitions (GC→AT) involved. Dibromonitromethane, a DBP that has not been tested for carcinogenicity, is also activated to a mutagen by a transgenic strain of Salmonella expressing GSTT1-1 (Kundu et al. 2004). Authors of both in vivo and in vitro studies have suggested a model whereby brominated THMs, after dermal absorption and inhalation, escape first-pass hepatic metabolism and reach target tissues in the urinary tract where the relative proportion of GSTT1 and oxidative enzymes is more favorable for GSTT1-mediated metabolism (Landi et al. 1999; Ross and Pegram 2003, 2004). In the bladder, the brominated THM could be activated to mutagens in a GSTT1+ person, leading to increased bladder cancer risk. Findings from experimental exposures to humans are also consistent with our observations. Exposure of humans to BDCM showed that 2 of 10 subjects, each of whom were GSTT1 null and had low CYP2E1 activity, had the lowest total BDCM metabolism (i.e., the highest blood levels of unmetabolized BDCM) and the highest peak levels of mutagenicity in their urine because of unmetabolized urinary BDCM (Leavens et al. 2007). In that experimental study, dermal exposure to BDCM resulted in blood levels 25–130 times higher than those from oral exposure, indicating that this is a major route of exposure to brominated THM. This is consistent with elevated risk for bladder cancer via showering, bathing, and/or swimming (Villanueva et al. 2007). Supporting our observation of a positive interaction of long-term DBPs with GSTT1+ is a finding of low risk among populations with the GSTT1 null genotype from an analysis of international data (Kim et al. 2002). Renal cell carcinoma risk is elevated among GSTT1+ individuals with occupational exposure to pesticides or to solvents such as trichloroethylene, possibly through a similar mechanism (Buzio et al. 2003; Karami et al. 2008).

The phase I metabolic enzyme CYP2E1 oxidizes a wide variety of alkanes, alkenes, and aromatic and halogenated hydrocarbons and activates many of them to carcinogenic compounds (Bolt et al. 2003). Several chemical species in the DBP mixture are potential substrates of CYP2E1. Our data, revealing a significant interaction between the level of long-term DBP exposure and the −1054C→T (sometimes designated as −1053C→T) rs2031920 SNP of CYP2E1, suggests the possibility of carcinogenic activation of one or more constituents of the DBP mixture by the common form of this enzyme. However, we make this observation cautiously. Although CYP2E1 levels are partially determined by genetic factors, the enzyme is highly inducible by alcohol and other factors, and its synthesis can be inhibited by food constituents (Oneta et al. 2002; Perocco et al. 2006). We were unable to control for these latter effects. The relatively small number of subjects with the rs2031920 CT/TT genotype (37 cases, 42 controls) contributed to the relative instability of this finding. In addition, our observation of an OR < 1.0 among the most highly exposed subjects with this genotype suggests a cautious interpretation of the CYP2E1 findings. It is unlikely that the aromatic amine substrates for NAT2, found in tobacco products and linked with bladder cancer, are present in the DBP mixture (Richardson 2003). However, DBP mixtures are complex, and we felt it worthwhile to evaluate the possibility of an interaction between DBPs and slow/rapid acetylation genotypes of NAT2 in risk of bladder cancer. Our expectation of little or no interaction was borne out in the data. The mechanism underlying associations between bladder cancer and the GSTM1 null genotype is not yet well understood (Garcia-Closas et al. 2005). Our data indicated that the strength of the association of bladder cancer with GSTM1 null is not affected by exposure to DBPs.

As our primary metric of exposure to DBPs, we used the long-term average THM concentration at the household level (Villanueva et al. 2007). DBPs are a complex mixture of halogenated organics whose composition and concentration vary in time and space. Hundreds of individual chemical species have been identified (Richardson 2003). THMs and HAAs are the chemical groups found at the highest concentrations in most mixtures, typically accounting for 20–30% of bound halogen. Although there is variability among the chemical components of DBP mixtures, the correlation coefficient between THMs and HAAs is usually > 0.7, and THM levels have been used in many epidemiologic studies as a surrogate for the full mixture. Although THMs and haloacetic acids are the most common chemical species within the DBP mixture, they may not be the most toxic/carcinogenic, and one or more of the polymorphisms of interest may be acting in important ways on other compounds with levels that are correlated with THMs.

Elevated levels of THMs and other DBPs are common in chlorinated swimming pools and cause elevated THM blood levels among swimmers (Aaggazzotti et al. 1998). We found no significant interaction of the polymorphisms studied here with ever use of swimming pools. However, the number of pool users was small, findings were not statistically stable, and the relative concentrations of various DBPs in pools differ from those in drinking water, precluding interpretation of these findings. In this hospital-based study, we had a high response rate among both cases and controls, and all diagnoses were histologically confirmed. Controls were matched to cases on age group, sex, and geographic area of residence at diagnosis. Although matching on area of residence may result in overmatching for type of water, possibly biasing relative risks toward the null, most hospital catchment areas were large enough to provide substantial variability in water sources. We examined residential history since 15 years of age, so that persons who may have had the same water source at the time of the study could easily have lived in other places previously. We conducted this analysis on a subset of the full study population, namely, subjects with genotype data and reliable information on DBP exposures for at least 70% of years between 15 years of age and diagnosis (cases) or interview (controls). We included 680 cases (of 1,219 in the full study) and 714 controls (of 1,271), and associations were mutually adjusted in a two-stage unconditional analysis. Exposure of humans to DBPs in drinking water, especially as a surrogate for the full mixture, typically accounting for 20–30% of bound halogen, is more favorable for GSTT1-mediated metabolism (Landi et al. 1999; Ross and Pegram 2003). Several chemical species have been identified (Richardson 2003). THMs and HAAs are the chemical groups found at the highest concentrations in most mixtures, typically accounting for 20–30% of bound halogen. Although there is variability among the chemical components of DBP mixtures, the correlation coefficient between THMs and HAAs is usually > 0.7, and THM levels have been used in many epidemiologic studies as a surrogate for the full mixture. Although THMs and haloacetic acids are the most common chemical species within the DBP mixture, they may not be the most toxic/carcinogenic, and one or more of the polymorphisms of interest may be acting in important ways on other compounds with levels that are correlated with THMs.

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Appendix I: Participating Study Centers in Spain
Institut Municipal d’Investigació Medica, Universitat Pompeu Fabra, Barcelona
Hospital del Mar, Universitat Autònoma de Barcelona, Barcelona
Hospital Germans Trias i Pujol, Badalona, Barcelona
Hospital de Sant Boi, Sant Boi de Llobregat, Barcelona
Consorti Hospitalari Parc Taulí, Sabadell Centre Hospitalari i Cardiològic, Manresa, Barcelona
Hospital Universitari de Canarias, La Laguna, Tenerife
Hospital Universitario Nuestra Señora de la Candelaria, Tenerife
Hospital General Universitario de Elche, Universidad Miguel Hernandez, Elche, Alicante
Universidad de Oviedo, Oviedo, Asturias
Hospital San Agustín, Aviles, Asturias
Hospital Central Covadonga, Oviedo, Asturias
Hospital Central General, Oviedo, Asturias
Hospital de Cabuernas, Gijon, Asturias
Hospital de Jove, Gijon, Asturias
Hospital de Cruz Roja, Gijon, Asturias
Hospital Alvarez-Buylla, Mieres, Asturias
Hospital Jarrío, Coana, Asturias
Hospital Carmen y Severo Ochoa, Cangas, Asturias

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