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Hair Dye Use and Risk of Bladder Cancer in the New England Bladder Cancer Study

Stella Koutros[1], **Debra T. Silverman**[1], **Dalsu Baris**[1], **Shelia Hoar Zahm**[1], **Lindsay M. Morton**[1], **Joanne S. Colt**[1], **David W. Hein**[2], **Lee E. Moore**[1], **Alison Johnson**[3], **Molly Schwenn**[4], **Sai Cherala**[5], **Alan Schned**[6], **Mark A. Doll**[2], **Nathaniel Rothman**[1], and **Margaret R. Karagas**[7]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD

[2]Department of Pharmacology & Toxicology, University of Louisville School of Medicine, Louisville, KY

[3]Vermont Cancer Registry, Burlington, VT

[4]Maine Cancer Registry, Augusta, ME

[5]New Hampshire Department of Health and Human Services Concord, NH

[6]Department of Pathology, Dartmouth Medical School, Hanover, NH

[7]Section of Biostatistics and Epidemiology Department of Community and Family Medicine, Dartmouth Medical School, Hanover, NH

Abstract

Aromatic amine components in hair dyes, and polymorphisms in genes that encode enzymes responsible for hair dye metabolism, may be related to bladder cancer risk. We evaluated the association between hair dye use and bladder cancer risk and effect modification by *NAT1*, *NAT2*, *GSTM1*, and *GSTT1* genotypes in a population-based case-control study of 1,193 incident cases and 1,418 controls from Maine, Vermont, and New Hampshire enrolled between 2001 and 2004. Individuals were interviewed in person using a computer-assisted personal interview to assess hair dye use and information on potential confounders and effect modifiers. No overall association between age at first use, year of first use, type of product, color, duration, or number of applications of hair dyes and bladder cancer among women or men was apparent but increased risks were observed in certain subgroups. Women who used permanent dyes and had a college degree, a marker of socioeconomic status, had an increased risk of bladder cancer (OR=3.3, 95% CI: 1.2, 8.9). Among these women, we found an increased risk of bladder cancer among exclusive users of permanent hair dyes who had *NAT2* slow acetylation phenotype (OR=7.3, 95% CI: 1.6, 32.6) compared to never users of dye with *NAT2* rapid/intermediate acetylation phenotype. While we found no relation between hair dye use and bladder cancer risk in women overall, we detected evidence of associations and gene-environment interaction with permanent hair dye use; however, this was limited to educated women. These results need confirmation with larger numbers, requiring pooling data from multiple studies.

Corresponding Author: Stella Koutros, Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., EPS 8115, MSC 7240, Rockville, MD 20852, USA., Phone: 301-594-6352, Fax: 301-402-1819, KoutrosS@mail.nih.gov.

Novelty and Impact: This study supports recent reports that permanent hair dyes may be most influential in bladder carcinogenesis. Genetic results also provide additional evidence for the role of the aromatic amine metabolizing enzyme *NAT2* as a modifier of the association between hair dyes and bladder cancer.

Keywords

hair dyes; bladder; cancer; aromatic amines; genetics

INTRODUCTION

Early occupational studies that identified aromatic amines as bladder carcinogens (1–2), as well as the discovery by Ames and colleagues that aromatic amine components of hair dyes were mutagenic (3), sparked several epidemiologic studies evaluating the risk of bladder cancer associated with hair dye use. Despite these observations, epidemiologic data to date on the potential association between hair dye use and bladder cancer risk are equivocal.(4–11) Three meta-analyses have concluded that there is no overall excess risk associated with personal use of hair dyes (12–14), and the International Agency for Research on Cancer Working Group considered that the available evidence for cancer of the bladder was overall 'inadequate' in their 2010 monograph.(15) However, controversy about whether hair dye use affects bladder cancer risk remains as some epidemiologic data indicate increased risk for use of specific types of hair dye. In particular, two studies reported an excess bladder cancer risk associated with permanent hair dyes (4;16), yet two others found no such association.(6;7)

One of the key components of permanent hair dyes is the aromatic amine p-phenylenediamine (PPD).(17) PPD has been found to be mutagenic in *Salmonella typhimurium* strain TA98 and to produce micronuclei in cultured human peripheral blood lymphocytes.(18) While there is debate about the carcinogenicity of PPD and of other components of permanent hair dyes, (19) Turesky and colleagues showed that the known bladder carcinogen, 4-aminobiphenyl (4-ABP) (20) has been present in black, red, and blonde commercial hair dyes and that PPD may be the source of this contamination.(21)

Additionally, *N*-acetylation is a major route of biotransformation of aromatic amine compounds, including those found in hair dyes. Two *N*-acetyltransferases, *N*-acetyltransferase-1 (NAT1) and *N*-acetyltransferase-2 (NAT2), have been well described in the metabolism of aromatic amines including PPD and 4-ABP.(22;23) Common polymorphisms in the genes that code for NAT1 and NAT2 result in variation in acetylation capacity.(22) In a population based case-control study in Los Angeles, exclusive permanent hair dye use was associated with a 2.9-fold risk of bladder cancer among *NAT2* slow acetylators, while no risk was observed among rapid acetylators (24). Genetic polymorphisms in glutathione S-transferases (*GSTM1* and *GSTT1)* also may affect the metabolism of constituents of hair dyes, but the biologic evidence to support that aromatic amine compounds are differentially metabolized by these enzymes and the role of *GSTT1* in bladder cancer susceptibility is unclear.(6;24) In light of these uncertainties, we evaluated the association between hair dye use and bladder cancer risk, as well as potential interactions between hair dye use and *NAT1, NAT2, GSTM1,* and *GSTT1* genotypes and other factors and bladder cancer risk, in a large population-based case-control study conducted in three states in northern New England.

MATERIAL & METHODS

Study Population

Details of the study population have been described previously.(25) Briefly, cases included all patients newly diagnosed with histologically confirmed carcinoma of the urinary bladder (including carcinoma *in situ*), aged 30–79 years among residents of Maine, Vermont, and New Hampshire. Cases were diagnosed between September 1, 2001 and October 31, 2004

(Maine and Vermont) or between January 1, 2002 and July 31, 2004 (New Hampshire) and were alive at the time of interview. For accurate and consistent classification of the cases, a re- review of the initial diagnostic slides to confirm diagnosis, histological classification, and tumor stage and grade was performed by an expert pathologist (A.S.). Of 1,878 eligible cases, 1,193 (65%) completed in-person interviews and were included in this analysis. Among eligible cases, the main reasons for nonparticipation included: refusal (19.4%), inability to locate the participant (1.5%) illness or death (12.1%), and inability to speak English (2.0%). Control subjects were frequency matched to cases by state, gender, and age at diagnosis (+/− 5 years) of cases. Control subjects aged 30 – 64 years were selected randomly from Department of Motor Vehicle (DMV) records in each state, and control subjects aged 65 – 79 years were selected from beneficiary records of the Centers for Medicare and Medicaid Services (CMS). This resulted in 1,418 (594 DMV and 824 CMS) interviewed control subjects (65% of eligible DMV and 65% of eligible CMS control subjects). Among eligible control subjects, reasons for nonparticipation included: refusal (21.3% of DMV and 21.4% of CMS), inability to locate the participant (8.1% of DMV and 3.1% of CMS%), illness or death (0.8% of DMV and 4.9% CMS), and inability to speak English (1.2% of DMV and 3.6% CMS).

Hair dye assessment

Individuals who agreed to participate were interviewed at home by a trained interviewer using a detailed computer-assisted personal interview. A standardized, structured questionnaire elicited demographic data and information on major known or suspected risk factors for bladder cancer, including hair dye use.(26) Ever use of hair coloring products was defined as use either at home or in a beauty salon for at least 5 times on hair, beard, mustache, or eyebrows. For each instance of use, information on age at first use, year of first use, type of product, color, duration, and number of applications was collected. To ascertain complete hair coloring histories, use of some products without dye components were assessed (bleach without color, highlights, henna) but excluded in ever/never use of hair dye analyses. Information on the type of product (permanent, semi-permanent, temporary, gradual, highlights, reverse highlights, bleach, gray or silver toner, henna, or other) was collected and a visual display card (10 pictures of hair by color: black, brown (dark, medium, light), red (dark,medium, light) and blonde (dark, medium, light)) was used to aide in the identification of hair dye color.

Genotyping and SNP selection

DNA for genotyping was extracted from exfoliated buccal cells collected from mouthwash samples using standard phenol-chloroform extraction methods. Genotyping analyses were successfully conducted on 1,088 of 1,171 (92.9%) cases and 1,282 of 1,418 (91.2%) controls who provided a mouthwash sample for genomic DNA extraction and genetic analyses. A total of 2,458 samples tested (94.9%) passed quality control and of these 2,370 were successfully genotyped (96.4%). Genotyped subjects did not differ by age, state, sex, or smoking characteristics from those included in the case-control study as a whole.

Genotypes were determined at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics of the National Cancer Institute (*GSTM1*, *GSTM2*) or the University of Louisville (*NAT1*, *NAT2*). For the *GSTM1*, *GSTT1* assays, melt curve/copy number assays [Applied Biosystems International, Foster City, CA, USA] were used to determine the number of deleted copies [0,1,2] of each gene. For *NAT1* and *NAT2* SNPs, a TaqMan assay [Applied Biosystems, Foster City, CA, USA] was used. Genotype assays used to determine acetylation status for *NAT1* were performed as previously described (27) and included the following SNPs: rs1057126, rs15561, rs4987076, rs4986782, rs5030839, rs56379106, rs56172717, and rs56318881. Genotype assays used to determine acetylation

status for *NAT2* included the following SNPs: rs1208, rs1799931, rs1041983, rs1801280, rs1799929, rs1799930, rs1805158. Descriptions and methods for *GSTM1* and *GSTM2* assays can be found at the National Cancer Institute SNP500Cancer website (snp500cancer.nci.nih.gov) and descriptions for NAT1 and NAT2 SNPs can be found at the University of Louisville, School of Medicine website (http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature). All genotypes were in Hardy-Weinberg equilibrium among the control population. Duplicate quality control samples showed 100% agreement for *NAT2*, *GSTM1*, and *GSTT1* and between 90–100% agreement for *NAT1* SNPs. Completion rates for *NAT2* SNPs were ≥99% except for rs1799931 which was 98%, >99% for all *NAT1* SNPs, and 98% and 93% for *GSTM1* and *GSTT1*, respectively.

Statistical Analysis

Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between hair dye use and bladder cancer risk. Ever use and usual use (most frequently used) by type and color were analyzed; usual color and usual product type were presented in tables. Product type was analyzed as permanent (includes permanent or reverse highlights), semi-permanent (includes semi-permanent or gray/silver toner), and temporary product use. Gradual and non-dye products were evaluated separately. Color was modeled as blonde, red, brown, or black, but was also parameterized in terms of 'depth of color' (light blonde, medium/dark blonde/light brown/light red, medium/dark brown/medium/dark red, and black) to reflect the fact that darker colors may have higher dye concentrations than lighter color products.(28) Similarly, additional categories for use of dark permanent dyes (thought to have higher hair dye concentrations than temporary or semi-permanent dyes (19;28) (permanent black, brown, and red)), and exclusive use of permanent dyes, were created and analyzed. Age at first use (<30, 31–45, >45 years), duration of use (<10, 10–19, 20–29, 30+ years), and number of applications (<50, 50–99, 100–199, 200+) were categorized for ease of comparison with similar studies on hair dyes and bladder cancer among women. Because of the low prevalence of hair dye use among men, we categorized hair dye use by tertiles or quartiles based on the control distribution in men. Year of first use and year of last use were defined to allow analysis of the timing of hair dye use relative to a reformulation of dye products that occurred in the 1970s and 1980s (17) (use before and after 1970 or 1980). Additional stratified analyses by state, product type, smoking (never, former, current), age at diagnosis/interview (≤60 or ≤65), and disease aggressiveness (i.e., low: grade1/2 and stage Ta/T0; high: grade 3+ or stage Tis/T1+) were performed among women. Because hair dye use is related to socioeconomic status (SES), we also stratified on education (no college degree, college degree) as a measure of SES.

We examined the association between hair dyes and bladder cancer stratified by genotype among ever users and permanent users with never users of hair dyes as the referent group. *GSTM1* and *GSTT1* genotypes were defined as null (−/−) if a deletion was present in both copies of the gene and active if one (+/−) or two (+/+) copies of the gene were present. The *NAT2* SNP results were used to assign the most likely functional acetylation genotypes previously identified and defined in human populations. Individuals homozygous for *NAT2* rapid-acetylator alleles were classified as having rapid-acetylator genotype/phenotype; individuals homozygous for slow-acetylator alleles were classified as having slow-acetylator genotype/phenotype, and heterozygous individuals (one rapid and one slow *NAT2* allele) were classified as having an intermediate-acetylator genotype/phenotype as previously described.(27;28) For analyses of *NAT1*, individuals with non-*NAT1*10* genotypes (*no NAT1*10* alleles) were compared with individuals with any *NAT1*10* genotypes (heterozygous or homozygous *NAT1*10*) since a previous study (23) reported an effect of

*NAT1*10* genotype on urinary bladder cancer risk following exposures to permanent hair dyes.

Hair dye models were adjusted for age (30–54, 55–64, 65–74, 75–79 years), race (White, Hispanic, other), state, and smoking status (never, occasional (25), former, current smoker), and genetic models were adjusted for age and race. Finer adjustment for smoking (duration) did not change risk estimates. Additional variables that were considered as potential confounders but were not included in our final models because they did not change risk estimates by more than 10% included high-risk occupation, urinary tract infection, use of non-steroidal anti-inflammatory drugs, education, multivitamin use, and water intake. Interactions were examined using a multiplicative model. The p-value for each interaction was computed by comparing nested models with and without the cross-product terms based on a likelihood ratio test. All statistical analyses were performed using SAS software version 9.1.3 (SAS Institute, Inc., Cary, NC).

RESULTS

Study participants were predominantly White, tended to be age 65 and older, and were most often from Maine (Table 1). Controls appeared to be slightly more educated than cases (percent with a college degree: 32.1% vs. 25.9% for men, 26.2% vs. 19.5% for women, respectively). As expected, a history of hair dye use was more common among women (56.4% for cases, 62.0% for controls) than men (5.4% for cases, 7.0% for controls).

Risk of bladder cancer associated with hair dye characteristics is presented by gender in Table 2. No association was apparent between ever use of hair dyes and bladder cancer among women (OR=0.7, 95% CI: 0.5, 1.0) or among men (OR=0.7, 95% CI: 0.5, 1.0). Compared with never users, neither color nor depth of color was associated with increased risk in either sex. Exclusive use of permanent hair dyes appeared unrelated to risk among women (OR=0.8, 95% CI: 0.5, 1.2) and men (OR=0.6, 95% CI: 0.3, 1.1). No excess risks of bladder cancer were observed with age or year at first use, duration, or number of applications of hair dyes compared with never users.

Within strata of product type (semi-permanent, permanent, dark permanent, or exclusive permanent) among women (Table 3), no increased risks were seen for any color type or depth of color category. A non statistically-significant elevated risk was observed for 30+ years of use of exclusive permanent hair dye compared to never use of hair dyes (OR=1.4, 95% CI: 0.7, 2.8) although no gradient in risk was apparent with increasing duration. No trends in risk were observed for age or year at first use, duration, or number of lifetime applications in any other product type strata.

We examined the association between hair dye use in women stratified by age at diagnosis/interview, state, product type, smoking, SES as measured by education, and by tumor subgroup (an indication of disease aggressiveness). No significant interactions were found between hair dye use in women and age at diagnosis/interview, state, product type, or smoking, nor did we detect differences by disease aggressiveness (data not shown). However, statistically significant interactions between education and several hair dye characteristics were observed (ever hair dye use P-interaction=0.012, product type P-interaction=0.0004, and age at first use P-interaction= 0.014, Table 4). Among women with a college degree, ever use of hair dyes was positively associated with bladder cancer risk (OR=1.9, 95% CI: 0.8, 4.4) compared with never users. Conversely, among women who had not obtained a college degree, there was a significant inverse association (OR=0.6, 95% CI: 0.4, 0.8). Women with a college degree who used permanent dyes had an increased risk of bladder cancer (OR=3.3, 95% CI: 1.2, 8.9), while women who used permanent dyes and did

not have a college degree had a decreased risk of bladder cancer (OR=0.5, 95% CI: 0.3, 0.7). Full demographic and hair dye use characteristics for female cases and controls in each education stratum are presented in Supplemental Table 1.

The association between *NAT1*, *NAT2*, *GSTM1*, and *GSTT1* genotype/phenotype and risk of bladder cancer for all women, women with no college degree, and women with a college degree is presented in Supplemental Table 2; there was no association between any genotype/phenotype and bladder cancer in any of these groups. We examined the interaction between hair dyes and genetic variation in *NAT1*, *NAT2*, *GSTM1*, and *GSTT1* among all women, women with no college degree, and women with a college degree (Table 5). None of the interactions between genetic variants and hair dye use were statistically significant (p-interaction>0.05, not shown). There were, however, significant increased risks in some subgroups with positive associations largely restricted to women with a college degree. Among women with a college degree, we found an increased risk of bladder cancer among exclusive users of permanent hair dyes who had *NAT2* slow acetylation phenotype (OR=7.3, 95% CI: 1.6, 32.6) compared to never users of dye with *NAT2* rapid/intermediate acetylation phenotype. Associations among women with a college degree did not differ by *NAT1* (non *NAT1**10 vs. any *NAT1**10) or *GSTM1* (any active vs. null) genotype. An increased risk of bladder cancer was observed among exclusive users of permanent dyes who had *GSTT1* active genotype (OR=5.9, 95% CI: 1.7, 20.0) while no association was observed among *GSTT1* null genotype. Genetic analyses adjusted for, or stratified by, smoking status showed similar results (data not shown).

DISCUSSION

In this population-based case-control study, we observed no association between ever use of hair dyes and bladder cancer among either women or men. Among women overall, there was no association between color, product type, age at first use, year of first use, or number of applications. In subgroup analyses of women with a college degree, use of permanent hair dyes was associated with a significant three-fold risk of bladder cancer, whereas less educated women (no college degree) experienced no increase risk. Genetic analyses showed an increased risk of bladder cancer among women who were exclusive users of permanent dyes and had NAT2 slow acetylation phenotype, but again only among those with a college degree.

The reason for differences in the magnitude and direction of the hair dye associations among highly educated and less educated women is unclear. Media reports linking hair dye use to cancer may have led to differential reporting between cases and controls (i.e., recall bias). Alternatively, more educated women may report their hair dye use more accurately, and thus results in this subgroup might better reflect the true odds ratios associated with hair dye use.(31) Another possibility is that more educated, and hence more affluent, women may go to salons for their hair dye applications and may therefore have been exposed to a different mixture of hair coloring products than women who personally applied over-the-counter products. Indeed, college educated women reported fewer lifetime applications of hair dyes than their less educated counterparts (Supplemental Table 1), which could reflect greater use of salons. Hairdressers are a known high-risk occupation for bladder cancer risk and professional-strength hair dyes are thought to be the exposures responsible.(1) Precise information on the potency of professional versus over–the- counter products to our knowledge has not been published. Further, although PPD is a main component of permanent hair dyes, we did not have information about what specific chemical might be contributing to the observed increased risks and thus whether exposure to specific chemical constituents of hair dyes differed by women’s educational status.

Our genetic results suggest that certain women may be particularly susceptible to the effects of hair dyes. *N*-acetylation by NAT2 in the liver is a recognized detoxification pathway in aromatic amine metabolism (30) and *NAT2* slow acetylator phenotype increasing urinary bladder cancer risk following aromatic amine exposure from cigarette smoke has also been described.(32) Although the interaction was not statistically significant, we observed an increased risk of bladder cancer primarily among exclusive users of permanent dyes who had NAT2 slow acetylation phenotypes compared to never users of dye with NAT2 rapid/intermediate acetylation phenotypes in females with a college degree. One study from Spain showed no modifying effect of *NAT2* genotype (6) while another, more comparable, California study showed a significant association between exclusive permanent hair dye use and bladder cancer in women with *NAT2* slow acetylator phenotype.(24) As in the California study, we did not observe a diminished effect of NAT2 slow phenotype after adjustment for NAT1, which is in linkage disequilibrium with NAT2 and thought to be the main detoxification pathway for hair dyes absorbed by the skin/scalp.(19) In our study, we found genetic modifiers of risk only among a subgroup of more educated women. Even if we suspect recall bias, it is unlikely that women would know their genotype. Thus the expected increase in risk among permanent and exclusive permanent users with *NAT2* slow acetylator phenotype supports a modifying role of *NAT2* genotype on the hair dye-bladder cancer association.

NAT1, *GSTM1*, and *GSTT1* genotypes did not appear to be important modifiers of the association between ever, permanent, or exclusive permanent use. Although there was an observed increased risk of bladder cancer associated with permanent hair dye use among college educated women with *GSTT1*-active genotypes compared to *GTTT1* null genotypes, the lack of evidence for the presence of GSTT1-metabolized conjugated mutagenic intermediates in hair dyes and the low prevalence of *GSTT1* null genotype indicates that this may be a chance association.

Several strengths of our study should be recognized. Our study is one of the largest case-control studies to evaluate the association between hair dyes and bladder cancer risk and includes a large number of exposed women in particular. It is also population-based and controlled for important confounders including smoking status. In an effort to minimize misclassification (28), subjects in this study used a visual display card to aide in the identification of hair color, a tool that had not been used in previous bladder cancer studies of hair dye use. In addition, high quality genotype information in these subjects allowed for the evaluation of effect modification by genotype or phenotype status.

Some limitations of our study should also be acknowledged. Numbers of subjects in stratified analyses were often small, resulting in imprecise estimates, particularly in genotype/phenotype subgroups. In college educated women, we observed an association between permanent dye use and bladder cancer; however, small numbers precluded estimation of exposure-response for frequency and duration of permanent dyes within this putative high susceptibility group. We also observed an inverse association among less educated women. Thus, the observed qualitative interaction between permanent hair dye use and education may suggest that the increased risk observed among college educated women could be due to chance. Similarly, these results need to be replicated to rule out the possibility of a false positive result from the multiple tests of interaction. Lastly, we cannot rule out the possibility of recall bias in our observed association between various hair dye use characteristics and bladder cancer risk within educational strata.

In summary, we observed no increased risk for hair dye use and risk of bladder cancer overall in women or men. We observed an increased risk for permanent hair dye use and exclusive permanent hair dye use among college educated women that will require

confirmation in other large studies. Genetic analyses of polymorphisms in enzymes known to influence aromatic amine-induced bladder cancer support the association between permanent hair dye use and bladder cancer risk in these women. Pooling data from studies with genetic information would provide greater statistical power to more definitively assess whether permanent hair dye use poses an increased risk of bladder cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PPD	p-phenylenediamine
4-ABP	4-aminobiphenyl
NAT1	*N*-acetyltransferase-1
NAT2	*N*-acetyltransferase-2
GSTM1	glutathione S-transferase Mu-1
GSTT1	glutathione S-transferase theta-1

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Table 1

Descriptive characteristics of bladder cancer cases and controls in northern New England.

Characteristic	Males		Females	
	Case n (%) n=911	Control n (%) n=1,039	Case n (%) n=282	Control n (%) n=379
Age				
<55	148 (16.3)	170 (16.4)	42 (14.9)	85 (22.4)
55–64	235 (25.8)	252 (24.3)	79 (28.0)	84 (22.2)
65–74	344 (37.8)	409 (39.4)	94 (33.3)	135 (35.6)
75+	184 (20.2)	208 (20.0)	67 (23.8)	75 (19.8)
Race/Ethnicity				
White	887 (97.4)	1004 (96.6)	273 (96.8)	367 (96.8)
Hispanic	17 (1.9)	17 (1.6)	6 (2.1)	7 (1.8)
Other	7 (0.8)	18 (1.7)	3 (1.1)	5 (1.3)
State				
Maine	456 (50.1)	557 (53.6)	128 (45.4)	183 (42.3)
Vermont	162 (17.8)	175 (16.8)	51 (18.1)	77 (20.3)
New Hampshire	293 (32.2)	307 (29.6)	103 (36.5)	119 (31.4)
Education				
No College Degree	675 (74.1)	706 (67.9)	227 (80.5)	280 (73.9)
College Degree	236 (25.9)	333 (32.1)	55 (19.5)	99 (26.2)
Ever used hair dye				
No	861 (94.5)	962 (92.6)	107 (37.9)	115 (30.3)
Yes	49 (5.4)	73 (7.0)	159 (56.4)	235 (62.0)
Color, No dye	1 (0.1)	4 (0.4)	16 (5.7)	28 (7.4)
Permanent dye				
Yes	18 (2.0)	28 (2.7)	86 (30.5)	123 (32.5)
Exclusive permanent dye				
Yes	18 (2.0)	28 (2.7)	72 (25.5)	100 (26.4)
Duration of dye use				
Never color	861 (94.5)	962 (92.6)	107 (37.9)	115 (30.3)
<10	35 (3.8)	48 (4.6)	68 (24.1)	87 (23.0)
10–19	8 (0.9)	15 (1.4)	30 (10.6)	69 (18.2)
20–29	4 (0.4)	5 (0.5)	24 (8.5)	38 (10.0)
30+	1 (0.1)	3 (0.3)	35 (12.4)	38 (10.0)
Mean	7.3	7.8	17.1	16.1
Lifetime applications				
Never color	861 (94.5)	962 (92.6)	107 (37.9)	115 (30.3)
<50	24 (2.6)	29 (2.8)	50 (17.7)	62 (16.4)
50–99	5 (0.6)	11 (1.1)	24 (8.5)	44 (11.6)

Characteristic	Males		Females	
	Case n (%) n=911	Control n (%) n=1,039	Case n (%) n=282	Control n (%) n=379
100–199	4 (0.4)	7 (0.7)	25 (8.9)	47 (12.4)
200+	6 (0.7)	6 (0.6)	44 (15.6)	63 (16.6)
Mean	90.2	78.0	176.9	190.9

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Table 2

Risk of bladder cancer and hair dye use characteristics in northern New England.

	Females		Males	
	Ca/Co	**OR* (95% CI)**	**Ca/Co**	**OR* (95% CI)**
Hair coloring product use				
Never	107/115	Ref	861/962	Ref
Ever	175/263	0.7 (0.5, 1.0)	50/77	0.7 (0.4, 1.0)
Hair dye use				
Never**	107/115	Ref	861/962	Ref
Ever	159/235	0.7 (0.5, 1.0)	49/73	0.7 (0.5, 1.0)
Dye color used most frequently				
Blonde	66/84	0.8 (0.5, 1.3)	5/9	0.6 (0.2, 1.8)
Red	22/50	0.4 (0.2, 0.8)	5/8	0.5 (0.2, 1.3)
Brown	55/81	0.7 (0.5, 1.2)	30/42	0.8 (0.5, 1.3)
Black	7/13	0.6 (0.2, 1.7)	4/6	--
Depth of color				
Light Blonde	49/56	0.9 (0.6, 1.6)	--	--
Medium/Dark Blonde, Light Brown, Light Red	40/63	0.7 (0.4, 1.1)	11/19	0.6 (0.3, 1.2)
Med/Dark Brown, Med/Dark Red	54/96	0.6 (0.4, 0.9)	37/37	0.8 (0.44, 1.3)
Black	7/13	0.6 (0.2, 1.7)	4/6	--
Product type				
Temporary	19/25	0.9 (0.4, 1.7)	6/7	0.9 (0.3, 2.6)
Semi-permanent	49/82	0.7 (0.4, 1.1)	17/21	0.8 (0.4, 1.6)
Permanent	86/123	0.7 (0.5, 1.1)	18/28	0.6 (0.3, 1.1)
Dark Permanent	45/84	0.6 (0.4, 1.0)	17/25	0.6 (0.3, 1.2)
Exclusive Permanent	72/100	0.8 (0.5, 1.2)	18/28	0.6 (0.3, 1.1)
Age at first use†				
T1	66/82	0.8 (0.5, 1.3)	11/27	0.4 (0.2, 0.8)
T2	56/87	0.7 (0.5, 1.2)	21/22	0.9 (0.5, 1.7)

	Females		Males	
	Ca/Co	**OR* (95% CI)**	**Ca/Co**	**OR* (95% CI)**
T3	37/66	0.6 (0.4, 1.0)	17/24	0.8 (0.4, 1.5)
Year first used				
< 1980	65/108	0.7 (0.5, 1.2)	38/53	0.8 (0.5, 1.2)
≥ 1980	94/127	0.7 (0.5, 1.1)	11/20	0.5 (0.2, 1.0)
Year last used				
< 1980	28/33	0.8 (0.4, 1.44)	4/4	--
1980–1989	11/21	0.5 (0.2, 1.14)	7/16	0.4 (0.2, 1.1)
1990–1999	21/25	0.8 (0.4, 1.52)	12/12	1.0 (0.5, 2.4)
2000+	97/153	0.7 (0.5, 1.10)	25/39	0.7 (0.5, 1.1)
Duration of use (years) ‡				
Q1	68/87	0.9 (0.55, 1.37)	11/22	0.5 (0.2, 1.0)
Q2	30/69	0.5 (0.27, 0.79)	15/15	1.1 (0.5, 2.3)
Q3	24/38	0.6 (0.33, 1.10)	14/18	0.7 (0.4, 1.6)
Q4	35/38	1.0 (0.56, 1.72)	8/16	0.6 (0.2, 1.3)
Number of lifetime applications§				
Q1	50/62	1.0 (0.6, 1.6)	8/14	0.5 (0.2, 1.2)
Q2	24/44	0.7 (0.4, 1.2)	14/13	1.0 (0.4, 2.2)
Q3	25/47	0.5 (0.3, 0.9)	7/13	0.5 (0.2, 1.3)
Q4	44/63	0.8 (0.5, 1.2)	10/13	0.9 (0.4, 2.2)

*Adjusted for age, race, state, and smoking.

**Never users of hair dye are the referent category for each run.

†Tertile 1(T1) female:≤30, Tertile 2 (T2) female: >30–45, Tertile 3(T3) female: >45; T1male:≤44, T2 male:>44–51, T3 male:>51

‡Q1female:<10, Q2 female:10–19, Q3 female:20–29, Q4 female:30+; Q1male:1, Q2 male:>1–4, Q3 male:>4–11, Q4 male:>11

§Q1 female:<50, Q2 female:50–99, Q3 female:100–199, Q4 female:200+; Q1 male:0–15, Q2 male:>15–41.4, Q3 male:>41.4–96.6, Q4 male:>96.6

Table 3

Risk of bladder cancer by product type among females in northern New England*

	Semi-Permanent		Permanent		Dark Permanent		Exclusive Permanent	
	Ca/Co	**OR** ** **(95% CI)**	**Ca/Co**	**OR** ** **(95% CI)**	**Ca/Co**	**OR** ** **(95% CI)**	**Ca/Co**	**OR** ** **(95% CI)**
Color								
Blonde	18/27	0.8 (0.4, 1.5)	47/56	0.8 (0.5, 1.3)	2/3	--	37/41	0.9 (0.5, 1.6)
Red	11/22	0.6 (0.2, 1.4)	14/34	0.4 (0.2, 0.9)	14/34	0.4 (0.2, 0.9)	11/22	0.5 (0.2, 1.2)
Brown	24/39	0.6 (0.3, 1.2)	28/41	0.7 (0.4, 1.3)	27/40	0.7 (0.4, 1.4)	20/29	0.8 (0.4, 1.5)
Black	4/5	--	2/7	--	2/7	--	1/6	--
Depth of color								
Light Blonde	14/14	1.2 (0.5, 2.9)	33/43	0.7 (0.4, 1.3)	2/2	--	28/32	0.9 (0.5, 1.6)
M/D Bl, L Br/R	16/30	0.6 (0.3, 1.2)	23/34	0.7 (0.4, 1.3)	9/21	0.5 (0.2, 1.1)	13/25	0.5 (0.2, 1.1)
M/D Brwn/Red	23/44	0.5 (0.3, 1.0)	33/54	0.7 (0.4, 1.1)	32/54	0.6 (0.4, 1.1)	27/35	0.9 (0.5, 1.6)
Black	4/5	--	2/7	--	2/7	--	1/7	--
Age at first use								
≤30	24/28	0.8 (0.4, 1.6)	45/58	0.7 (0.4, 1.3)	17/34	0.5 (0.3, 1.0)	33/38	0.8 (0.4, 1.5)
>30–45	22/36	0.7 (0.4, 1.4)	35/56	0.7 (0.3, 1.1)	20/35	0.7 (0.4, 1.3)	27/41	0.7 (0.4, 1.3)
>45	14/32	0.5 (0.2, 1.0)	14/26	0.6 (0.3, 1.2)	8/15	0.6 (0.2, 1.4)	12/21	0.7 (0.3, 1.5)
Year first used								
< 1980	36/41	0.8 (0.5, 1.5)	60/86	0.6 (0.4, 1.0)	23/49	0.5 (0.3, 0.8)	45/61	0.7 (0.4, 1.1)
≥ 1980	24/55	0.5 (0.3, 1.0)	34/54	0.8 (0.4, 1.5)	22/35	0.9 (0.4, 1.8)	27/39	0.9 (0.5, 1.7)
Duration of use								
<10	27/4	0.8 (0.4, 1.4)	37/41	1.0 (0.5, 1.8)	21/25	1.1 (0.5, 2.3)	31/31	1.1 (0.6, 2.1)
10–19	13/30	0.5 (0.2, 1.1)	15/42	0.3 (0.2, 0.7)	7/23	0.3 (0.1, 0.8)	10/30	0.3 (0.1, 0.7)
20–29	11/10	1.2 (0.4, 3.1)	13/29	0.4 (0.2, 0.9)	3/18	0.2 (0.1, 0.6)	10/21	0.4 (0.2, 1.0)
30+	9/15	0.5 (0.2, 1.4)	29/27	1.1 (0.6, 2.1)	14/18	0.8 (0.4, 1.7)	21/17	1.4 (0.7, 2.8)
Lifetime applications								
<50	21/33	0.7 (0.4, 1.4)	28/29	1.3 (0.7, 2.6)	20/20	1.4 (0.6, 3.1)	23/22	1.5 (0.7, 3.3)
50–99	8/19	0.5 (0.2, 1.4)	15/28	0.6 (0.3, 1.3)	7/15	0.6 (0.2, 1.7)	11/20	0.6 (0.3, 1.4)

	Semi-Permanent		Permanent		Dark Permanent		Exclusive Permanent	
	Ca/Co	OR** (95% CI)	Ca/Co	OR** (95% CI)	Ca/Co	OR** (95% CI)	Ca/Co	OR** (95% CI)
100–199	13/18	0.6 (0.3, 1.4)	14/29	0.4 (0.2, 0.8)	3/19	0.1 (0.04, 0.5)	8/23	0.3 (0.1, 0.7)
200+	15/21	0.8 (0.4, 1.8)	28/42	0.7 (0.4, 1.2)	14/29	0.5 (0.2, 1.0)	22/26	0.9 (0.4, 1.7)

* Never users of hair dye are the referent category for each run.

** Adjusted for age, race, state, and smoking.

Table 4

Risk of bladder cancer stratified by education among females in northern New England.

Education level	No College Degree		College Degree		p-interaction
	Ca/Co	OR* (95% CI)	Ca/Co	OR* (95% CI)	
Hair dye use					
Never**	88/75	Ref	19/40	Ref	
Ever	126/185	0.6 (0.4, 0.8)	33/50	1.9 (0.8, 4.4)	0.012
Color					
Blonde	54/66	0.7 (0.4, 1.1)	12/18	2.0 (0.7, 6.1)	
Red	19/40	0.4 (0.2, 0.7)	3/10	0.8 (0.2, 4.3)	
Brown	41/64	0.6 (0.3, 1.0)	14/17	1.8 (0.7, 5.0)	
Black	6/9	0.6 (0.2, 1.9)	1/4	--	0.214
Depth of color					
Light Blonde	38/45	0.7 (0.4, 1.2)	11/11	3.4 (1.0, 11.9)	
M/D Bl, L Br/R	37/51	0.6 (0.4, 1.1)	3/12	0.5 (0.2, 3.3)	
M/D Brwn/Red	39/74	0.4 (0.2, 0.7)	15/22	1.8 (0.6, 4.9)	
Black	6/9	0.6 (0.2, 1.9)	1/4	--	0.078
Product type					
Temporary	18/22	0.7 (0.4, 1.5)	1/3	--	
Semi-permanent	43/57	0.7 (0.4, 1.1)	6/25	0.7 (0.2, 2.3)	
Permanent	61/102	0.5 (0.3, 0.7)	25/21	3.3 (1.2, 8.9)	0.0004
Dark Permanent	30/70	0.4 (0.2, 0.6)	15/14	2.8 (0.9, 8.7)	0.0006
Exclusive Permanent	50/87	0.5 (0.4, 0.7)	22/13	4.9 (1.7, 14.6)	<0.0001
Age at first use					
≤30	58/67	0.7 (0.4, 1.1)	8/15	1.5 (0.4, 5.0)	
>30–45	39/70	0.5 (0.3, 0.8)	17/17	3.2 (1.1, 9.2)	
>45	29/48	0.6 (0.3, 1.0)	8/18	1.1 (0.3, 3.5)	0.014
Year of first use					
< 1980	79/108	0.5 (0.3, 0.9)	15/19	2.0 (0.8, 5.2)	

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Education level	No College Degree		College Degree		p-interaction
	Ca/Co	OR* (95% CI)	Ca/Co	OR* (95% CI)	
≥ 1980	47/77	0.6 (0.3, 1.0)	18/31	1.8 (0.6, 4.9)	
Duration of use					
<10	52/68	0.7 (0.4, 1.2)	16/19	2.0 (0.7, 5.9)	
10–19	24/46	0.4 (0.2, 0.8)	6/23	0.7 (0.2, 2.3)	
20+	49/69	0.5 (0.3, 0.9)	10/7	3.8 (1.1, 13.0)	0.070
Lifetime applications					
<50	36/49	0.7 (0.4, 1.3)	14/13	2.8 (0.9, 9.0)	
50–99	19/27	0.6 (0.3, 1.3)	5/17	0.9 (0.2, 3.8)	
100+	59/94	0.5 (0.3, 0.8)	10/16	1.5 (0.5, 4.4)	0.116

* Adjusted for age, race, state, smoking.

** Never users of hair dye are the referent category for each model

Table 5

Joint effects of hair dyes and phenotype or genotype and risk of bladder by education strata

ALL WOMEN	**NAT2 Rapid/Intermediate**		**NAT2 slow**	
Hair dye use	**Ca/Co**	**OR* (95% CI)**	**Ca/Co**	**OR* (95% CI)**
Never	33/36	Ref	55/62	1.0 (0.5, 1.8)
Ever	59/84	0.8 (0.4, 1.4)	86/124	0.8 (0.4, 1.4)
Permanent	27/48	0.6 (0.3, 1.2)	51/61	0.9 (0.5, 1.6)
Exclusive permanent	21/41	0.6 (0.3, 1.1)	44/46	1.0 (0.5, 1.9)
WOMEN NO COLLEGE DEGREE	**NAT2 Rapid/Intermediate**		**NAT2 slow**	
Never	25/23	Ref	46/41	1.0 (0.5, 2.1)
Ever	48/67	0.7 (0.3, 1.3)	66/96	0.6 (0.3, 1.2)
Permanent	20/39	0.4 (0.2, 1.0)	35/49	0.6 (0.3, 1.3)
Exclusive permanent	15/34	0.4 (0.2, 0.9)	29/40	0.6 (0.3, 1.3)
WOMEN COLLEGE DEGREE	**NAT2 Rapid/Intermediate**		**NAT2 slow**	
Never	8/13	Ref	9/21	0.7 (0.2, 2.4)
Ever	11/17	1.3 (0.4, 4.4)	20/28	1.4 (0.5, 4.4)
Permanent	7/9	1.7 (0.4, 7.0)	16/12	3.1 (0.9, 11.3)
Exclusive permanent	6/7	2.1 (0.5, 9.5)	15/6	7.3 (1.6, 32.6)
ALL WOMEN	**non NAT1*10**		**any NAT1*10**	
Hair dye use				
Never	53/64	Ref	34/34	1.2 (0.7, 2.2)
Ever	109/148	0.9 (0.6, 1.4)	36/55	0.8 (0.5, 1.5)
Permanent	54/74	0.9 (0.5, 1.5)	24/34	0.8 (0.4, 1.6)
Exclusive permanent	46/60	0.9 (0.5, 1.6)	20/28	0.8 (0.4, 1.7)
WOMEN NO COLLEGE DEGREE	**non NAT1*10**		**any NAT1*10**	
Never	42/41	Ref	29/24	1.2 (0.6, 2.4)
Ever	90/116	0.8 (0.5, 1.3)	25/44	0.6 (0.3, 1.1)
Permanent	40/62	0.6 (0.3, 1.1)	15/27	0.5 (0.2, 1.1)
Exclusive permanent	33/52	0.6 (0.3, 1.1)	12/24	0.5 (0.2, 1.1)
WOMEN COLLEGE DEGREE	**non NAT1*10**		**any NAT1*10**	
Never	11/23	Ref	5/10	1.0 (0.3, 3.7)
Ever	19/32	1.5 (0.6, 3.9)	11/11	2.7 (0.8, 9.0)
Permanent	14/12	3.0 (1.0, 9.3)	9/7	3.8 (1.0, 14.9)
Exclusive permanent	13/8	4.9 (1.4, 17.3)	8/4	6.3 (1.5, 36.5)
ALL WOMEN	**GSTM1 Any Active**		**GSTM1 Null**	
Hair dye use				
Never	35/39	Ref	49/59	0.9 (0.5, 1.7)
Ever	55/89	0.7 (0.4, 1.3)	85/110	0.9 (0.5, 1.5)
Permanent	29/37	0.9 (0.4, 1.7)	48/66	0.8 (0.4, 1.5)

ALL WOMEN	**NAT2 Rapid/Intermediate**		**NAT2 slow**	
Hair dye use	**Ca/Co**	**OR* (95% CI)**	**Ca/Co**	**OR* (95% CI)**
Exclusive permanent	22/34	0.7 (0.3, 1.5)	43/50	0.9 (0.5, 1.8)
WOMEN NO COLLEGE DEGREE	**GSTM1 Any Active**		**GSTM1 Null**	
Never	30/27	Ref	37/37	0.9 (0.5, 1.8)
Ever	43/68	0.6 (0.3, 1.1)	69/90	0.7 (0.4, 1.3)
Permanent	21/32	0.5 (0.3, 1.2)	35/52	0.6 (0.3, 1.1)
Exclusive permanent	15/30	0.4 (0.2, 1.0)	30/42	0.6 (0.3, 1.2)
WOMEN COLLEGE DEGREE	**GSTM1 Any Active**		**GSTM1 Null**	
Never	5/12	Ref	12/22	1.3 (0.4, 4.7)
Ever	12/21	1.7 (0.5, 6.5)	16/20	2.3 (0.6, 8.3)
Permanent	8/5	4.6 (0.9, 22.5)	13/14	2.8 (0.7, 11.2)
Exclusive permanent	7/4	5.2 (1.0, 27.9)	13/8	5.4 (1.2, 24.0)
ALL WOMEN	**GSTT1 Any Active**		**GSTT1 Null**	
Hair dye use				
Never	70/79	Ref	15/14	1.2 (0.5, 2.7)
Ever	109/151	0.9 (0.6, 1.3)	21/45	0.5 (0.3, 1.0)
Permanent	60/81	0.8 (0.5, 1.3)	10/26	0.4 (0.2, 1.0)
Exclusive permanent	50/62	0.9 (0.5, 1.5)	10/22	0.5 (0.2, 1.2)
WOMEN NO COLLEGE DEGREE	**GSTT1 Any Active**		**GSTT1 Null**	
Never	56/51	Ref	12/10	1.1 (0.4, 2.7)
Ever	86/118	0.7 (0.4, 1.1)	17/35	0.4 (0.2, 0.9)
Permanent	42/66	0.5 (0.3, 0.9)	8/21	0.3 (0.1, 0.8)
Exclusive permanent	33/53	0.5 (0.3, 1.0)	8/19	0.4 (0.1, 0.9)
WOMEN COLLEGE DEGREE	**GSTT1 Any Active**		**GSTT1 Null**	
Never	14/28	Ref	3/4	1.6 (0.3, 8.3)
Ever	23/33	1.7 (0.7, 4.2)	4/10	1.0 (0.3, 4.0)
Permanent	18/15	3.3 (1.1, 9.8)	2/5	1.0 (0.2, 6.2)
Exclusive permanent	17/9	5.9 (1.7, 20.0)	2/3	1.9 (0.3, 13.9)

*Adjusted for age, race