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Original Article

Genetic association between the *COMT* genotype and urinary levels of tea polyphenols and their metabolites among daily green tea drinkers

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Abstract: Available *in vitro* and animal studies have shown cancer protective effects of tea polyphenols. Recent study suggests a greater protective effect of green tea intake on breast cancer risk among women possessing the low-activity associated genotype of the catechol-*O*-methyltransferase (*COMT*), which may modulate the metabolism and excretion of tea polyphenols through urine. To determine the effect of *COMT* genotype on urinary excretion of tea polyphenol metabolites of daily green tea drinkers, a cross-sectional analysis was performed within the Shanghai Cohort Study, a population-based, prospective investigation of diet and cancer in 18,244 men. In addition to an in-person interview, each participant provided a blood and urine sample at baseline. In the present study, *COMT* genotype (rs4680) and five urinary metabolites of tea polyphenols were determined in 660 cohort subjects who self-identified as daily drinkers of green tea. All urinary tea polyphenol measurements were expressed in units of urinary creatinine. Men possessing the homozygous low-activity associated *COMT* genotype (*LL*) exhibited statistically significantly lower urinary levels of individual as well as all of the five tea polyphenol metabolites under study relative to individuals possessing the wild type high-activity associated *COMT* genotype (*HH*) or the heterozygous variant genotype (*HL*). Levels of urinary tea polyphenol metabolites were comparable between men possessing the *HH* and *HL* genotypes. The present study demonstrated that men carrying low-activity associated *COMT* genotype excreted less tea polyphenols from urine, which suggests that they may retain more tea polyphenols in their bodies and derive greater health benefits from green tea intake.

Keywords: Urinary tea polyphenols, green tea, *COMT* genotype

Introduction

Tea, one of the most widely consumed beverages in the world, is brewed from dried leaves of the plant *Camellia Sinensis*. There are two major types of tea – green tea and black tea (also called red tea in certain parts of the world). Both green and black teas are made of the leaves of the same kind of plants but using different processing approaches. For green tea, fresh tea leaves are steamed or heated immediately after harvest, resulting in minimal oxidation of the naturally occurring polyphenols in the tea leaves. On the other hand, in the processing of black tea, tea leaves are dried and crushed upon harvesting to encourage oxidation, which converts the indigenous tea polyphenols (primarily catechins and gallocatechins) to other polyphenols (mainly theaflavins and thearubigens). Tea preparations have inhibitory activities against tumorigenesis in many experimental studies [1]. The chemoprotective compounds in tea preparations are believed to be tea polyphenols, in particular, (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), epicatechin (EC), and (-)-epicatechin-3-gallate (ECG). The bioavailability and biotransformation of tea polyphenols are key factors

limiting the anti-tumorigenesis activities of tea catechins *in vivo*, and explain in part the inconsistent associations between green tea intake and cancer risk reported in the epidemiologic literature [2-6].

Methylation represents a major metabolic pathway for tea polyphenols. Experimental studies have shown that several catechol-containing tea polyphenols, particularly EGC, are rapidly *O*-methylated, which is catalyzed by catechol-*O*-methyl transferase (COMT), an enzyme ubiquitously present in humans [7, 8]. In the regard, one would speculate that individuals possessing a genotype resulting in reduced COMT activity would have lower capacity in COMT-catalyzed methylation of tea polyphenols and their urinary excretion relative to those with the genotype encoding high-activity COMT.

It is known that *COMT* is polymorphic. A single G to A transition at codon 158 of *COMT* (rs4680) results in an amino acid change from valine to methionine in the cytosolic/membrane-bound form of COMT. This amino acid change leads to a 3- to 4-fold decrease in enzymatic activity *in vitro* [9, 10]. Individuals possessing the homozygous variant genotype (*LL*) showed approximately 60% lower COMT activity than those possessing the homozygous wild-type genotype (*HH*)[11]. Individuals with the heterozygous genotype (*HL*) exhibited intermediate levels of enzymatic activity. In a case-control study of breast cancer among Asian women of Los Angeles County, we observed a protective effect of green tea intake that was mainly confined to women with one or more copies of the low-activity associated *COMT* allele [12].

To date, there are no direct data on the possible influence of *COMT* genotype on the metabolism and excretion of tea catechins in humans. We conducted a cross-sectional analysis within the Shanghai Cohort Study to evaluate whether urinary levels of tea catechin metabolites differ significantly between subjects possessing the low- versus high-activity associated genotypes of *COMT* among daily drinkers of green tea.

Subjects and methods

The Shanghai Cohort Study

The design of the Shanghai Cohort Study has been described in detail previously [13, 14]. Briefly, between January 1, 1986 and September 30, 1989, all eligible male residents of 4 small, geographically defined communities over a wide area of the city of Shanghai were invited to participate in a prospective epidemiologic investigation of diet and cancer. The eligibility criteria were ages 45 to 64 years and no history of cancer. During the 3-year recruitment period, 18,244 men (representing approximately 80% of eligible subjects) were enrolled in the study. At recruitment, each participant was interviewed in person by a trained nurse using a structured questionnaire asking for information on demographic characteristics, histories of tobacco and alcohol use, usual dietary habits and medical history. This study had been approved by the Institutional Review Boards of the University of Minnesota and the Shanghai Cancer Institute. Written informed consent was obtained from all subjects.

At recruitment, each participant was asked whether he had ever drunk alcoholic beverages at least once a week for 6 months or more. If the answer was yes, he was asked to provide the typically consumed amounts of beer, wine and spirits separately. One drink was defined as 360 g of beer (12.6 g of ethanol), 103 g of wine (12.3 g of ethanol) or 30 g of spirits (12.9 g of ethanol). Smokers were identified as men who smoked at least 1 cigarette per day for 6 months or more. Information about current smoking status (yes or no), the number of cigarettes smoked per day, and number of years of smoking over lifetime was obtained from all ever smokers. For those who had quit the smoking habit, the number of years since quitting smoking was recorded. Current diet was assessed via a food frequency questionnaire that included 45 food groups or items representing commonly consumed local foods. The methods for computing selected dietary nutrients were previously described [15].

At the completion of the baseline interview, a 10-mL non-fasting blood sample and a single-void urine sample were collected from each participant. The collection of biospecimens from study subjects usually took place between 5 pm and 9 pm, on average about 3 hr after the last meal. Following collection, the urine sample was immediately placed in an ice box and transported on the same day to the processing laboratory where it was stored at 4˚C overnight. The following morning, three aliquots (two with 10

ml each, and one with 25 ml) of urine sample per subject were made and stored at -20˚C until 2001 when they were transferred to -70˚C freezers until analysis.

Information on subjects' tea consumption patterns was not collected during the baseline interview. Detailed questions regarding tea intake over lifetime were asked during the annual in-person follow-up interviews of 2001 and 2002. Each subject was asked if he had ever drunk tea beverages at least once a week for 6 months or more. All regular tea drinkers were further asked about age at starting to drink tea regularly, number of years of regular tea drinking, average amount of dry tea leaves used per week or per month and type of tea (green tea, black tea, or oolong tea) consumed. Responses were obtained from 14,210 (98%) of 14,531 surviving cohort members. Fifty-four percent of the cohort participants drank any type of tea on a daily basis. Virtually all (95%) of these daily tea drinkers were exclusive green tea drinkers.

Beginning on September 1, 2001, we asked all surviving cohort members (N=14,531) to donate a buccal cell sample for DNA analysis. Of the 14,210 subjects who provided tea intake status, 13,816 (95% of total survival cohort members) subjects provided buccal cell samples by the end of the 2-year effort.

Study subjects

A series of nested case-control studies of cancer using urine- and/or blood-based biomarkers as exposure indices had been conducted within this cohort database. In each of these studies, multiple cohort controls per index case of cancer patient were selected such that the controls were individually matched to the index case by age (within 2 years), neighborhood of residence, and date of biospecimen collection (within 6 months). Subjects for the present study were selected from all control participants (n=1,881) of the colorectal cancer [16] and hepatocellular carcinoma [17] studies. Intact urine samples were available for all of these control subjects in storage as of December 31, 2001. Of the 1,881 control subjects, all daily green tea drinkers were included (n=774). Compared with daily green tea drinkers that were in the cohort but not included in the present analysis (n=6,100), these 774 daily green tea drinkers were slightly older (56.3 versus 53.8 years at baseline) and attained lower level of education (19% versus 22% with college education or above), but otherwise comparable in terms of cigarette smoking (60% versus 61% current smokers), alcohol drinking (50% versus 49%), and consumption of green tea (8.3 versus 8.4 gram/day).

Laboratory analyses

All urine samples were identified by unique codes that are devoid of subjects' personal details. Five metabolites of tea catechins in urine were assayed: EGC, EC, methylepigallocatechin (4'-MeEGC), 5-(3',4',5'- trihydroxyphenyl)-γ-valerolactone (M4), and 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (M6) using previously validated high-performance liquid chromatography (HPLC) method, which allows the determination of free and conjugated forms of tea catechins in plasma, urine, and other biological samples [18, 19]. The urine samples were incubated with β-D-glucuronidase and sulfate at 37˚C for 45 min to hydrolyze the catechin conjugates. Following hydrolysis, the urine samples were directly extracted with ethyl acetate. The samples were then centrifuged and injected onto the HPLC. The tea catechins were analyzed using an ultraviolet/visible (UV/VIS) detector and electrochemical detector. EGCG is not detectable in urine, and thus it is not included in the present analysis. All assays were performed in a single batch. Urinary creatinine (Cr) concentration was determined on each sample using a modified method as described previously [20]. The within-batch coefficients of variations on duplicate samples were 11.7% for EGC, 11.2% for 4'-MeEGC, 7.9% for EC, 24.6% for M4, 7.4% for M6 and 5.8% for creatinine.

DNA was extracted from buccal cells using a standard method described previously [21, 22]. A genotyping assay for the *COMT* genotype (rs4680) was developed using the fluorogenic 5'-nuclease (TaqMan) platform [23, 24]. The assays were performed using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The oligonucleotide primers for amplification of the polymorphic region of *COMT* were GC009for (5'-GCCATCACCCAGCGGAT-3') and GC009rev (5'-AACGGGTCAGGCATGCAC-3'). In addition, the fluorogenic oligonucleotide probes used to detect each of the alleles were GC009F (5'-GATTTCGCTGGCATGAAGGACAAG-3') labeled with 6-FAM to detect the L allele and

GC009C (5'-GATTTCGCTGGCGTGAAGGACAAG-3') labeled with CY3 to detect the *H* allele (BioSearch Technologies, Novato, CA). PCR amplification using about 10 ng of genomic DNA was performed in a thermal cycler (MWG Biotech, High Point, NC) with an initial step of 95°C for 10 min followed by 50 cycles of 95°C for 25 s and 62°C for 1 min. The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System and the results analyzed with Sequence Detection Software (Applied Biosystems). Urinary EGC and 4'-MeEGC were undetectable in 39 urine samples. DNA samples were unavailable on another 75 subjects. Therefore, the final sample size of this study was 660.

Statistical analysis

The concentrations of tea polyphenols (EGC and EC) and metabolites of tea polyphenols (4'-MeEGC, M4, and M6) in urine were expressed in units of urinary creatinine by weight (μmol/g Cr) to take into account the varying water contents across individual spot urine samples. For each subject, we summed EGC, EC, 4'-MeEGC, M4, and M6 in micromoles to create a 'total tea polyphenols' index. The distributions of all urinary biomarkers were markedly skewed toward high values. Therefore, formal statistical testing was performed on logarithmically transformed values, and geometric (as opposed to arithmetic) means of all urinary tea polyphenol biomarkers are presented.

Green tea drinkers were classified as low (<5 g of dry tea leaves per day), intermediate (5-<10 g of dry tea leaves per day), or high (10 or more g of dry tea leaves per day) consumers. Five g of dry tea leaves equate to approximately 1 to 2 cups of tea. Because urinary tea polyphenol levels were measured at one time point, we used the analysis of variance (ANOVA) fixed effect model to compare levels of urinary measurements across discrete categories of demographic/lifestyle variables (Table 1), and the chi-square test to compare the distributions of the *COMT* genotype in the study population by the level of green tea consumption (Table 2). The multivariable regression method was used to compare levels of urinary measurements across the 3 categories of *COMT* genotype (Table 3). Covariates included in the multivariable regression model included: age (continuous), current number of cigarettes smoked per day (none, < 20 cigarettes/d, ≥ 20 cigarettes/d), and current number of alcoholic drinks consumed per day (none, < 2 drinks/d, 2 - < 4 drinks/d, ≥ 4 drinks/d). Statistical analyses were performed using the SAS version 9.1 (SAS Institute Inc., Cary, NC) statistical software package. All reported *p* values are two-sided and $p < 0.05$ was considered statistically significant.

Results

The mean age of our subjects was 56.7 years (standard deviation (SD), 5.0). The mean daily intake of green tea was 8.3 g (SD = 5.0 g) of dry tea leaves, or 3-4 cups per day. Twenty-three percent had no formal education or primary school education only, while 26% had completed some college education. Sixty percent were current smokers of cigarettes whereas 49% currently consumed alcoholic beverages on a weekly basis.

Table 1 shows the geometric means of individual and summed urinary metabolites of tea catechins by selected demographic and lifestyle factors. There is a highly statistically significant association between self-reported levels of green tea drinking (g of dry leaves per day) and levels of the five individual tea catechins and their summed total ($p < 0.01$). Cigarette smokers displayed higher levels of total tea polyphenols ($p < 0.01$) relative to nonsmokers. Level of education and alcohol consumption were unrelated to levels of tea catechins in urine.

The genotypic distribution of *COMT* among study subjects was: *HH,* 52.0%; *HL,* 40.6%. and *LL,* 7.4%. The prevalence of the *L* allele was 27.7%. The distribution of the *L* allele was in agreement with the Hardy-Weinberg equilibrium ($p = 0.74$). The frequency distribution of *COMT* genotype was unrelated to subjects' levels of green tea consumption (**Table 2**).

Compared with those carrying at least one copy of high-activity associated *COMT* allele (*HH* and *HL* genotypes), men with the homozygous low-activity associated (*LL)* genotype of *COMT* had a statistically significant or borderline statistically significant reduction in levels of tea catechins in urine (**Table 3**). Relative to those with the *HH* genotype, the levels of urinary tea catechins were lower by 35-45% among men carrying the *LL* genotype. Levels of urinary tea catechins were comparable between men possessing the

Table 1. Mean levels of urinary total tea polyphenols and their metabolites (μmol/g Cr) by selected demographic and lifestyle factors*

	No. of subjects (%)	EGC	4'-MeEGC	EC	M4	M6	Total tea polyphenols§
Level of education							
No or primary school	150 (22.7)	7.7	23.6	2.3	2.6	29.6	106.0
Middle school	340 (51.5)	7.0	24.3	2.1	3.3	37.2	105.6
College or above	170 (25.8)	6.7	28.1	2.4	3.4	32.9	103.7
p for trend†		0.45	0.30	0.77	0.23	0.67	0.89
Current cigarette smoking							
Never or former smokers	265 (40.2)	6.0	24.0	2.1	2.3	26.8	84.0
Current smokers	395 (59.8)	7.9	25.8	2.3	3.9	40.3	122.3
P†		0.03	0.55	0.37	< 0.01	< 0.01	< 0.01
Never or former smokers	265 (40.2)	6.0	24.0	2.1	2.3	26.8	84.0
< 20 cigarettes/day	196 (29.7)	8.1	26.0	2.4	3.7	40.8	126.6
≥ 20 cigarettes/day	199 (30.1)	7.7	25.7	2.2	4.1	39.8	118.2
p for trend†		0.08	0.62	0.57	< 0.01	0.02	< 0.01
Alcohol drinking							
Non-drinkers	334 (50.6)	7.0	25.9	2.1	2.8	33.5	104.1
Drinkers	326 (49.4)	7.1	24.2	2.3	3.6	35.0	106.3
P†		0.87	0.57	0.60	0.10	0.77	0.85
Non-drinkers	334 (50.6)	7.0	25.9	2.1	2.8	33.5	104.1
< 2 drinks/day	182 (27.6)	7.0	26.2	2.1	2.9	33.9	105.5
2 - < 4 drinks/day	90 (13.6)	7.0	21.4	2.4	3.3	29.5	95.5
≥ 4 drinks/day	54 (8.2)	7.9	22.7	2.3	6.9	47.3	120.4
p for trend†		0.63	0.31	0.59	< 0.01	0.48	0.79
Dry tea leaved consumed (g/day)							
< 5	131 (19.8)	5.0	19.8	1.6	1.9	19.9	65.7
5 - < 10	367 (55.6)	6.8	24.2	2.2	3.2	33.8	107.1
≥ 10	162 (24.6)	9.9	32.8	2.7	4.4	54.1	147.7
p for trend†		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

* All geometric means were derived from analysis of covariance with age (years) as a covariate.
§ The sum of EGC, EC, 4'-MeEGC, M4 and M6.
† *P*-values were derived from analysis of covariance with adjustment for age.
Abbreviations: EGC: (-)-epigallocatechin, 4'-MeEGC: methylepigallocatechin (4'-MeEGC), EC: epicatechin, M4: 5-(3',4',5'- trihydroxyphenyl)-γ-valerolactone, M6: 5-(3',4'-dihydroxyphenyl)-γ-valerolactone

Table 2. *COMT* genotype frequency by green tea intake

	HH, n (%) (n = 343)	*HL*, n (%) (n = 268)	*LL*, n (%) (n = 49)	*p**
Green tea leaves consumed (g/day)				
< 5	61 (17.8)	62 (23.1)	8 (16.3)	0.18
5 - < 10	187 (54.5)	152 (56.7)	28 (57.2)	
≥ 10	95 (27.7)	54 (20.2)	13 (26.5)	

* A *p*-value was derived from chi-square test.

Table 3. Geometric mean (95% confidence interval) levels of urinary total tea polyphenols and their metabolites by the *COMT* genotype*

	COMT genotype			*p*†
Urinary tea polyphenol metabolites	*HH* (n = 343)	*HL* (n = 268)	*LL* (n = 49)	
EGC (μmol/g Cr)	7.3 (5.8 – 9.2)	7.3 (5.7 – 9.3)	4.5 (2.6 – 7.3)	0.047
4'-MeEGC (μmol/g Cr)	25.2 (20.1 – 31.6)	21.8 (17.2 – 27.6)	15.0 (9.2 – 23.8)	0.049
EC (μmol/g Cr)	2.3 (1.8 – 2.8)	2.2 (1.8 – 2.8)	1.5 (0.8 – 2.3)	0.057
M4 (μmol/g Cr)	4.0 (3.1 – 5.1)	4.0 (3.0 – 5.1)	2.2 (1.2 – 3.9)	0.037
M6 (μmol/g Cr)	33.7 (25.3 – 44.7)	36.8 (27.4 – 49.3)	21.7 (11.9 – 38.7)	0.099
Total tea polyphenols (μmol/g Cr)	105.0 (85.3 – 129.0)	102.6 (82.8 – 127.2)	58.7 (38.4 – 89.6)	0.007

* Geometric means were derived from analysis of covariance with the following covariates: age (year), cigarette smoking (nonsmokers, < 20 cigarettes/day, and ≥20 cigarettes/day), alcohol drinking (nondrinkers, <2 drinks/day, 2- <4 drinks/day, and ≥4 drinks/day), and amount of tea leaves consumed (< 5, 5 - < 10, and ≥ 10 g/day).

† *P*-values were derived from multivariate regression models that compared the *LL* genotype with the *HL* and *HH* genotypes combined with the adjustment for all variables listed above.

Abbreviations: EGC: (-)-epigallocatechin, 4'-MeEGC: methylepigallocatechin (4'-MeEGC), EC: epicatechin, M4: 5-(3',4',5'- trihydroxyphenyl)-γ-valerolactone, M6: 5-(3',4'-dihydroxyphenyl)-γ-valerolactone

HH and *HL* genotypes (*p* values ranged 0.24–0.99).

We further examined the *COMT*-urinary tea polyphenol association stratified by levels of green tea consumption. Among low consumers (<5 g/day of dry green tea leaves), men carrying the *HL* or *LL* genotype showed statistically significant 35-45% reduction in urinary levels of 4'-MeEGC and EC than those with the *HH* genotype of *COMT.* On the other hand, the urinary excretion rates of tea catechins and their metabolites were comparable between individuals possessing the high-activity and low-activity associated *COMT* genotypes when consuming greater amount of green tea (≥5 g/day) (data not shown). In addition, we examined the potential modifying effects of amounts of green tea drunk, cigarette smoking, or alcohol drinking on the *COMT* genotype-urinary tea catechins associations. Results were null (data not shown).

Discussion

In the present study, among daily drinkers of green tea, urinary levels of specific metabolites of tea catechins differ significantly between subjects possessing the low- versus high-activity associated genotypes of *COMT*. Subjects possessing the homozygous low-activity associated (*LL) COMT* genotype showed a statistically significant, 35-45% reduction in urinary levels of tea polyphenol metabolites relative to their *HH* genotype counterparts who consumed comparable amounts of green tea. To our knowledge, this is the first epidemiologic study implicating a modulating effect of the *COMT* genotype on urinary excretion of tea polyphenols in humans. The findings of the present study are consistent with, and provide a biological rationale for the stronger protective effect of tea consumption on breast cancer risk in Asian women possessing the low-activity associated *COMT* genotype (*LL* or *HL*) versus their high-activity associated *COMT* genotype (*HH*) counterparts in Los Angeles [12]. The prevalence of the *L* allele in the present study population was 27.7%. This prevalence was in agreement with the reported data in Chinese women in Los Angeles (27.1%) [12]. In the previous study, the *HL* and *LL* genotypes were combined and compared with the *HH* genotype because of the small sample sizes in these categories. In the present study, the urinary levels of tea catechin metabolites were comparable between men carrying the *HL* genotype and those with *HH* genotype. Our results suggest the significant impact of *COMT* genotype on the efficiency of metabolism and excretion of tea catechin metabolites through urine may be confined to individuals carrying the *LL* genotype.

A recent case-control study of breast cancer in Shanghai, China, reported that regular green tea intake was inversely associated with risk of breast cancer but this relationship did not vary according to women's *COMT* genotype [25]. The inconsistent findings of that study with those observed in Asian women in Los Angeles [12] could be due to the difference in tea consumption between the two populations. The differential effect of the *COMT* genotype on the inverse tea-breast cancer risk association could be masked by high exposure to tea polyphenols in the former study population given the moderate effect of the gene on tea catechin metabolism. The findings of the present study that the *COMT* genotype had impact on urinary excretion of tea catechins and their metabolites in individuals with low consumption of green tea (<5 g/day) support the explanation for the discrepancy of previous study findings between the populations with high and low tea consumption.

O-methylation, catalyzed by COMT, is the major metabolism pathway of tea polyphenols. The other three excretion pathways of tea polyphenols are glucuronidation, sulfation, and ring-fission metabolism [26]. COMT catalyzes the transfer of methyl groups from S-adenosylmethionine (SAM) to one of the hydroxyl groups of catechol and produces *O*-methylated catechol and S-adenosyl-L-homocysteine (SAH) [27, 28]. Among the three metabolites (4'-MeEGC, M4, and M6) of tea polyphenols measured, 4'-MeEGC is a methylated metabolite of EGC. The other two, M4 and M6, are the ring-fission metabolites of EGC and EC, respectively, which are believed to be formed in the colon by microflora [26]. We found decreased excretion of all of these three metabolites as well as unmetabolized EGC and EC among men carrying the *LL* genotype. Given the metabolic balance and compensation, one would expect an increased efficiency of glucuronidation, sulfation, and ring-fission metabolism when the *O*-methylation descreases among individuals carrying the *LL COMT* genotype. However, the lower excretion of M4 and M6 observed in men with the *LL COMT* genotype in

the present study does not suggest the enhancement of the ring-fission metabolism in these individuals. Since the laborary assays for specific sulfation and glucuronidation products of tea catechins are under development, we were unable to quantify the urinary sulfated and glucuronidated tea catechins in our study subjects. Future studies are warranted to simultaneously quantify and examine the metabolites of tea polyphenols by all pathways that will shed light on the understanding of the metabolism of tea polyphenols and their *in vivo* effect.

The present study has several strengths. The study population has a high prevalence of green tea drinking habits with a wide range in the amounts drunk per day. The collection of urine samples took place between 5 pm and 9 pm, on average about 3 hours after intake of the last meal. Pharmacokinetic studies have shown that plasma concentrations of EGCG, EGC and EC reach peak levels between 1.5 and 3.0 hr after ingestion of tea or tea polyphenols in humans [29, 30]. Maximum urinary excretion of EGC and EC normally occurs at 2–6 hr after tea ingestion and most is excreted within 8–9 hr following exposure [29]. The urinary excretion of 4'-MeEGC occurs around the same time as EGC excretion [31]. In Shanghai, it is common among adults to drink tea after a meal, especially dinner. Thus, the levels of urinary tea polyphenols captured in the present study likely represent their near peak levels during a typical day.

One concern of the present study is the possible degradation of test compounds in urine over time. No data are available regarding the effect of long-term storage on the concentrations of urinary tea polyphenols, although prior study has shown that these urinary measurements remained unchaned when samples had been stored at -80°C for 6 months [32]. However, possible degradation of test compounds in urine over time should not affect our conclusion concerning the difference among the three *COMT* genotypes since the durations of urine storage were comparable. The means (standard deviations) of urine storage from their collection to laboratory analyses for individuals with the *HH, HL,* and *LL* genotype were 17.1 (0.95), 17.1 (0.90), and 16.8 (0.62) years, respectively (P = 0.37). The present study population consists solely of male subjects. The reduced urinary levels of tea polyphenols and their metabolites were primarily seen among men with the *LL* genotype. These results seemed not in agreement with the results of the case-control study of breast cancer in women, in which the protective effect of tea drinking on breast cancer risk was seen in both women with the *LL* and *HL* genotypes. Given lack of data on a potential gender difference in the bioavailability and biotransformation of tea polyphenols, further studies are warranted to clarify whether gender would modify the interplay between the *COMT* genotype and tea intake on beneficial effects of tea polyphenols.

In conclusion, men carrying low-activity associated *COMT* genotype excreted significantly less amount of tea polyphenols and their metabolites from urine compared with high-activity associated *COMT* genotypes even when they consumed comparable amount of green tea. This study suggests that individuals with the low-activity associated *COMT* genotype may retain more tea polyphenols in their bodies and derive greater health benefits from green tea intake. The findings of this study are consistent with, and provide a biological rationale for our prior observation that tea intake protects against breast cancer development among women possessing the low-activity associated *COMT* genotype seem to derive greater benefits from green tea drinking against breast cancer development.

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