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***GSTM1* Null Genotype Associated with Age-standardized Cancer Mortality Rate in 45 Countries from Five Continents: An Ecologic Study**

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Abstract: To evaluate the public health impact of association between prevalence of *GSTM1* and *GSTT1* null genotypes and age-standardized cancer mortality rate the present study, using data of 45 countries from five continents was done. Data of prevalence of *GSTM1* and *GSTT1* null genotypes was obtained from published articles in scientific journals. Data about the age-standardized mortality rates due to cancers (per 100,000 population) (for 2002) and total expenditure on health at international dollar rate per capita (for 2003) were obtained from the World Health Organization Web site <http://www.who.int>. In order to rule out the possible confounding effect of total expenditure on health per capita on the mortality rate, partial correlation analysis was carried out. After controlling the total expenditure on health per capita, significant positive correlation between prevalence of *GSTM1* null genotype and age-standardized cancer mortality rate was observed ($r = 0.301$, $df = 42$, $p = 0.047$).

Key words: *GSTM1*, *GSTT1*, age-standardized mortality rate, total expenditure on health per capita, ecologic study

INTRODUCTION

Glutathione S-transferases (GSTs) are a group of enzymes known to play an important role in the detoxification of several endogenous and exogenous toxic and carcinogenic substances. In human GST enzymes are divided into several classes including mu and theta. *GSTM1* (a member of class mu) and *GSTT1* (a member of class theta) products catalyze the conjugation of glutathione to a number of chemicals present in cigarette smoke including epoxide derivatives of polycyclic aromatic hydrocarbons, the main carcinogens found in tobacco smoke, methylating agents, pesticides, industrial solvents and reactive oxygen species.

Allelic polymorphisms in the *GSTM1* and *GSTT1* genes have been defined. The functional consequences of the *GSTM1* and the *GSTT1* null genotypes are obvious in terms of enzymes activity: no gene, no enzymes and no activity. The disease-association studies were conducted in different populations for various cancers (Engel *et al.*, 2002; Garcia-Closas *et al.*, 2005; Hashibe *et al.*, 2003; La Torre *et al.*, 2005; Saadat 2006; Sull *et al.*, 2004; Tripathy and Roy 2006; Ye *et al.*, 2006; Ye and Parry, 2003; Ye and Song, 2005).

Members of the GST family catalyze detoxification of many alkylating agents, including nitrosoureas, platinum compounds and melphalan. They may also detoxify the free radicals formed by chemotherapy drugs and radiation (Cholon *et al.*, 1992; Dulik *et al.*, 1986; Lien *et al.*, 2002). Several studies support the idea that active *GSTM1* and *GSTT1* enzymes may improve cancerous patients after chemotherapy (Beeghly *et al.*, 2006; Sweeney *et al.*, 2003; Goto *et al.*, 1996). To evaluate the public health impact of prevalence of *GSTM1* and *GSTT1* null genotypes on mortality due to cancers, the association between prevalence of the null-genotypes of GSTs and age-standardized mortality rates due to cancers in 45 countries from five continents was analyzed.

MATERIALS AND METHODS

Data of prevalence of *GSTM1* and *GSTT1* null genotypes was obtained from published articles in scientific journals (Table 1).

Table 1: Prevalence of *GSTT1* and *GSTM1* null genotypes, total expenditure on health per capita and age-standardized cancer mortality rates of the countries

Country	Age-standardized cancer mortality rate (per 100,000 population)	Total expenditure on health at international dollar rate per capita	Null genotype (%)		References
			<i>GSTT1</i>	<i>GSTM1</i>	
Argentina	142	1067	11.0	45.0	Moore <i>et al.</i> , 2004
Australia	127	2874	16.3	45.7	Chenevix-Trench <i>et al.</i> , 1995; Curran <i>et al.</i> , 2000
Belgium	148	2828	16.1	51.4	Kellen <i>et al.</i> , 2006
Brazil	142	597	20.7	35.8	Arruda <i>et al.</i> , 2001; Canalle <i>et al.</i> , 2004; Colombo <i>et al.</i> , 2004; da Fonte de Amorim <i>et al.</i> , 2002; Gaspar <i>et al.</i> , 2002; Morari <i>et al.</i> , 2002; Rossini <i>et al.</i> , 2002
Bulgaria	125	573	16.1	51.8	Toncheva <i>et al.</i> , 2004
Canada	138	2989	19.9	53.8	Casson <i>et al.</i> , 2003; Hamel <i>et al.</i> , 2000; He <i>et al.</i> , 2004; Krajinovic <i>et al.</i> , 1999
Chile	137	707	-	22.6	Alberti <i>et al.</i> , 1996; Quinones <i>et al.</i> , 1999, 2001
China	148	278	46.6	55.8	Cai <i>et al.</i> , 2001; Chan <i>et al.</i> , 2005; Chen <i>et al.</i> , 2004; Egan <i>et al.</i> , 2004; Gao <i>et al.</i> , 2002; Guo <i>et al.</i> , 1996; Lan <i>et al.</i> , 2000; Li <i>et al.</i> , 2005; London <i>et al.</i> , 2000; Mu <i>et al.</i> , 2005; Nan <i>et al.</i> , 2005; Setiawan <i>et al.</i> , 2000; Tan <i>et al.</i> , 2000; Zhong <i>et al.</i> , 2006; Wang <i>et al.</i> , 2003
Colombia	117	522	14.6	37.5	Torres <i>et al.</i> , 2004
Czech Republic	177	1302	18.9	50.6	Holla <i>et al.</i> , 2006; Sarmanova <i>et al.</i> , 2000
Denmark	167	2762	11.9	53.9	Christiansen <i>et al.</i> , 2006; Knudsen <i>et al.</i> , 1999
Egypt	84	235	27.1	52.3	Abdel-Rahman <i>et al.</i> , 1996, 1999, 2001; Anwar <i>et al.</i> , 1996; Hamdy <i>et al.</i> , 2003
Estonia	150	682	17.9	53.0	Juronen <i>et al.</i> , 2000; Mikelsaar <i>et al.</i> , 1994; Tasa <i>et al.</i> , 1996
Finland	115	2108	12.4	46.4	Hirvonen <i>et al.</i> , 1993; Mitrunen <i>et al.</i> , 2001; Piirila <i>et al.</i> , 2001; Saarikoski <i>et al.</i> , 1998; Voho <i>et al.</i> , 2006
France	142	2902	15.6	48.6	Abbas <i>et al.</i> , 2004; Jourenkova <i>et al.</i> , 1997, 1998; Jourenkova-Mironova <i>et al.</i> , 1999a, 1999b; Maugard <i>et al.</i> , 1998; Stucker <i>et al.</i> , 2000
Gambia	144	96	43.8	27.3	Kirk <i>et al.</i> , 2005
Germany	141	3001	17.3	50.9	Brockmoller <i>et al.</i> , 1993; Gronau <i>et al.</i> , 2003; Haase <i>et al.</i> , 2002; Hahn <i>et al.</i> , 2002; Jahnke <i>et al.</i> , 1996; Kabesch <i>et al.</i> , 2004; Ko <i>et al.</i> , 2001; Matthias <i>et al.</i> , 1998; Risch <i>et al.</i> , 2003; Schneider <i>et al.</i> , 2004; Westphal <i>et al.</i> , 2000
Greece	132	1997	10.5	47.6	Dialyna <i>et al.</i> , 2001, 2003; Tsaouri <i>et al.</i> , 2004
Hungary	201	1269	13.8	47.0	Kiss <i>et al.</i> , 2000; Schoket <i>et al.</i> , 2001; Tuimala <i>et al.</i> , 2002
Iceland	136	3110	20.5	54.2	Gudmundsdottir <i>et al.</i> , 2001
India	109	82	16.7	23.7	Buch <i>et al.</i> , 2002; Chacko <i>et al.</i> , 2005;

Table 1: Continue

Country	Age-standardized cancer mortality rate(per 100,000 population)	Total expenditure on health at international dollar rate per capita	Null genotype (%)		References
			<i>GSTT1</i>	<i>GSTM1</i>	
					Girisha <i>et al.</i> , 2004; Mishra <i>et al.</i> , 2004; Mittal <i>et al.</i> , 2004; Nair <i>et al.</i> , 1999; Sharma <i>et al.</i> , 2006; Sobti <i>et al.</i> , 2004, 2005, 2006; Sreelekha <i>et al.</i> , 2001, Srivastava <i>et al.</i> , 2004
Iran	113	498	38.2	40.6	Kohan <i>et al.</i> , 2006; Saadat <i>et al.</i> , 2004a, 2004b, 2004c; Saadat and Saadat 2000, 2001, 2003a, 2003b; Saadat and Mohabatkar 2004; Sepehr <i>et al.</i> , 2004; Mohammadzadeh Ghobadloo <i>et al.</i> , 2004
Italy	134	2266	17.9	49.8	Alberti <i>et al.</i> , 1996; Buzio <i>et al.</i> , 2003; D'Alo <i>et al.</i> , 2004; Fustinoni <i>et al.</i> , 2002; Palli <i>et al.</i> , 2005; Pavanello <i>et al.</i> , 2002; Sgambato <i>et al.</i> , 2002
Japan	119	2244	48.5	48.9	Harada <i>et al.</i> , 2001; Hayashi <i>et al.</i> , 1992; Inoue <i>et al.</i> , 2000; Katoh <i>et al.</i> , 1995, 1996; Kihara <i>et al.</i> , 1997; Morita <i>et al.</i> , 1999; Naoe <i>et al.</i> , 2000; Nimura <i>et al.</i> , 1997; Sasai <i>et al.</i> , 1999; Sata <i>et al.</i> , 2003; Sato <i>et al.</i> , 1999; Sekine <i>et al.</i> , 1995; Tanimoto <i>et al.</i> , 1992
Mexico	88	582	5.1	39.4	David <i>et al.</i> , 2003; Gallegos-Arreola <i>et al.</i> , 2004; Romieu <i>et al.</i> , 2004
Netherlands	155	2987	20.3	47.1	Luchtenborg <i>et al.</i> , 2005; Oude Ophuis <i>et al.</i> , 1998; van Delft <i>et al.</i> , 2001
Norway	137	3809	17.4	46.8	Nedelcheva Kristensen <i>et al.</i> , 1998; Ryberg <i>et al.</i> , 1997
Paraguay	141	301	17.9	35.8	Gaspar <i>et al.</i> , 2002
Poland	180	745	17.3	49.8	Butkiewicz <i>et al.</i> , 1999; Gajicka <i>et al.</i> , 2005; Gawronska-Szklarz <i>et al.</i> , 1999; Lan <i>et al.</i> , 2001
Portugal	140	1791	25.5	53.5	Krajinovic <i>et al.</i> , 2002; Lemos <i>et al.</i> , 1999; Moreira <i>et al.</i> , 1996
Republic of Korea	169	1074	52.0	52.7	Cho <i>et al.</i> , 2005; Choi <i>et al.</i> , 2003; Hong <i>et al.</i> , 1998; Hong <i>et al.</i> , 2000; Hur <i>et al.</i> , 2005; Jong Jeong <i>et al.</i> , 2003; Lee <i>et al.</i> , 2002; Park <i>et al.</i> , 2002; Yim <i>et al.</i> , 2000
Russia	152	551	20.4	45.2	Ivaschenko <i>et al.</i> , 2001, 2002; Korytina <i>et al.</i> , 2005; Vakhitova <i>et al.</i> , 2001
Saudi Arabia	109	578	8.7	15.4	Abu-Amero <i>et al.</i> , 2006
Singapore	128	1156	42.6	47.6	Gago-Dominguez <i>et al.</i> , 2004; Lee <i>et al.</i> , 1998; Seow <i>et al.</i> , 2002; Zhao <i>et al.</i> , 1995, 2001
Slovakia	170	777	18.3	50.7	Dusinska <i>et al.</i> , 2001; Habalova <i>et al.</i> , 2004; Salagovic <i>et al.</i> , 1998, 1999; Somorovska <i>et al.</i> , 1999
Spain	131	1853	22.2	49.4	Garcia-Closas <i>et al.</i> , 2005; Gonzalez <i>et al.</i> , 1998; Hernandez <i>et al.</i> , 2003; Ladero <i>et al.</i> , 2006; Ruano-Ravina <i>et al.</i> , 2003; To-Figueras <i>et al.</i> , 1997
Sudan	112	54	37.8	38.8	Tiemersma <i>et al.</i> , 2001
Sweden	116	2704	14.0	54.0	Alexandrie <i>et al.</i> , 1994, 2004
Tanzania	151	29	25.0	33.0	Dandara <i>et al.</i> , 2002
Thailand	129	260	47.2	30.2	Kietthubthew <i>et al.</i> , 2001
Turkey	95	528	22.5	36.7	Balta <i>et al.</i> , 2003; Ozturk <i>et al.</i> , 2003; Pinarbasi <i>et al.</i> , 2003, 2005; Tamer <i>et al.</i> , 2004, 2005; Toruner <i>et al.</i> , 2001

Table 1: Continue

Country	Age-standardized cancer mortality rate (per 100,000 population)	Total expenditure on health at international dollar rate per capita	Null genotype (%)		References
			<i>GSTT1</i>	<i>GSTM1</i>	
UK	143	2389	15.6	50.6	Allan <i>et al.</i> , 2001; Zhong <i>et al.</i> , 1993; Basu <i>et al.</i> , 1997; Deakin <i>et al.</i> , 1996; Fryer <i>et al.</i> , 2000; Lewis <i>et al.</i> , 2002; Loktionov <i>et al.</i> , 2001; Rollinson <i>et al.</i> , 2000; Ye <i>et al.</i> , 2002; Seedhouse <i>et al.</i> , 2004; Welfare <i>et al.</i> , 1999
Ukraine	139	305	14.2	50.6	Ebrahimi <i>et al.</i> , 2004
USA	134	5711	20.4	44.7	Abdel-Rahman <i>et al.</i> , 2001; Barnette <i>et al.</i> , 2004; Chen <i>et al.</i> , 1997; Cheng <i>et al.</i> , 1999; Cote <i>et al.</i> , 2005; Crump <i>et al.</i> , 2000; Davies <i>et al.</i> , 2000, 2002; Ford <i>et al.</i> , 2000; Gertig <i>et al.</i> , 1998; Gilliland <i>et al.</i> , 2002; Hakim <i>et al.</i> , 2004; Hanna <i>et al.</i> , 2001; Helzlsouer <i>et al.</i> , 1998; Kelada <i>et al.</i> , 2000; Li <i>et al.</i> , 2001; McWilliams <i>et al.</i> , 2000; Nazar-Stewart <i>et al.</i> , 2003; Olshan <i>et al.</i> , 2000; Park <i>et al.</i> , 1997; Roodi <i>et al.</i> , 2004; Wrensch <i>et al.</i> , 2004; Slattery <i>et al.</i> , 1998; Sweeney <i>et al.</i> , 2000; Trizna <i>et al.</i> , 1995; Woo <i>et al.</i> , 2000
Zimbabwe	122	132	-	30.0	Mukanganyama <i>et al.</i> , 1997

Since cancers are more common in older age groups, a population that is older will have a higher crude incidence rate. Age-standardized mortality rate is a procedure where weighted averages of age-specific rate are used to modify rates to a standard population in order to minimize the effects of differences in the age composition of given populations (such as provinces or census divisions) when comparing rates for these populations. The purpose of this rate is to compare groups of people from different backgrounds and age structures. The age-standardized rates for both sexes combined also adjust for possible differences in the gender distribution. Here we used the age-standardized cancer mortality rates for both sexes. Data about the age-standardized mortality rates due to cancers (per 100,000 population) (for 2002) and total expenditure on health at international dollar rate per capita (for 2003) were obtained from the World Health Organization Web site <http://www.who.int>.

Inclusion criteria were availability of data about total expenditure on health at international dollar rate per capita, age-standardized mortality rates due to cancers and prevalence of the GSTs genotypes of the country.

Correlations between the variables were determined using Pearson's correlation coefficient analysis. Also the partial correlation coefficient analysis was done. Statistical analysis was performed using SPSS (version 11.5) statistical software package. p-value less than 0.05 considered statistically significant.

RESULTS AND DISCUSSION

There is significant positive correlation between age-standardized cancer mortality rate and the prevalence of *GSTM1* null genotype ($r = 0.327$, $df = 43$, $p = 0.028$). However, there is no significant correlation between age-standardized cancer mortality rate and the prevalence of *GSTT1* null genotype ($r = -0.01$, $df = 41$, $p = 0.977$).

It is now widely accepted that there is significant differences between populations for *GSTM1* and *GSTT1* null genotypes (Engel *et al.*, 2002; Garcia-Closas *et al.*, 2005; Hashibe *et al.*, 2003; La Torre *et al.*, 2005; Saadat 2006; Sull *et al.*, 2004; Tripathy and Roy 2006; Ye and Parry, 2003; Ye and Song, 2005; Ye *et al.*, 2006). As it is appeared from Table 1, the frequency of *GSTT1* null genotype in European populations is about 10-22% which is increased from north to the south. In Asian

populations both *GSTT1* and *GSTM1* null genotypes increased from west to the east. Therefore, the frequencies of *GSTM1* and *GSTT1* null genotypes showed geographical distributions. On the other hand, total expenditure on health per capita also showed a geographical pattern with highest mean value among European populations. Taken together, in order to show the actual correlation between prevalence of the null genotypes (*GSTM1* and *GSTT1*) and age-standardized cancer mortality rate and rule out the possible confounding effect of total expenditure on health per capita on the mortality rates, partial correlation analysis was carried out. After controlling the total expenditure on health per capita, significant positive correlation between prevalence of *GSTM1* null genotype and age-standardized mortality rate due to cancers was observed ($r = 0.301$, $df = 42$, $p = 0.047$). However, there is no significant correlation between age-standardized cancer mortality rate and the prevalence of *GSTT1* null genotype after controlling for total expenditure on health per capita ($r = 0.028$, $df = 40$, $p = 0.860$). This finding is in the same direction as that reported by several investigators from different populations in the cancers association studies (Engel *et al.*, 2002; Garcia-Closas *et al.*, 2005; Hashibe *et al.*, 2003; La Torre *et al.*, 2005; Saadat 2006; Sull *et al.*, 2004; Tripathy and Roy 2006; Ye and Parry, 2003; Ye and Song, 2005; Ye *et al.*, 2006). There have been a number of studies of GST genetic polymorphisms and outcomes in several types of cancers, such as breast, lung, colorectal and ovarian cancers (Beeghly *et al.*, 2006; Goto *et al.*, 1996; Okcu *et al.*, 2004; Stoehlmacher *et al.*, 2002; Sweeney *et al.*, 2003; Yang *et al.*, 2005). Several studies demonstrated that the survival in cancerous patients with *GSTM1* null genotype was shorter than that in patients with active genotype of *GSTM1* (Beeghly *et al.*, 2006; Sweeney *et al.*, 2003; Goto *et al.*, 1996; Okcu *et al.*, 2004). Our present results indirectly, confirmed these studies. Some other studies, however, have reported no relationship or opposite association between *GSTM1* polymorphism and survival (Stoehlmacher *et al.*, 2002; Yang *et al.*, 2005).

From the present data, it might be concluded that prevalence of *GSTM1* null genotype influences cancer mortality rate independent of age and sex structure of the populations, the total expenditure on health per capita and the prevalence of *GSTT1* null genotype. Based on the present finding about 9% ($r^2 = 0.301^2$) of differences between age-standardized cancer mortality rate between countries might be interpreted by the prevalence of *GSTM1* null genotype. It seems that the *GSTM1* deficiency accounts relatively high level of cancer mortality, because of the high prevalence of *GSTM1* null genotype. The attributed risk should be estimated in the future studies.

It should be mentioned that the present study has some limitations. First of all, the present study is an ecological study and has limitation of ecological studies. Second, total expenditure on health per capita is not the target confounding variable per se. So, there would be a certain degree of residual confounding still present in the calculated partial correlation coefficients. Third age-standardized mortality rate and total expenditure on health per capita for the study countries was not related to a same year.

Finally, case-control and cohort studies investigating relationship between genetic polymorphisms of *GSTM1* and cancer mortality may confirm the present preliminary data.

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