Effect of cruciferous vegetables on lung cancer in patients stratified by genetic status: a mendelian randomisation approach

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Whether consumption of cruciferous vegetables protects against lung cancer is unclear, largely because of potential confounding factors. We therefore studied the role of cruciferous vegetables in lung cancer after stratifying by GSTM1 and GSTT1 status, two genes implicated in the elimination of isothiocyanates, the likely chemopreventative compound. In 2141 cases and 2168 controls, weekly consumption of cruciferous vegetables protected against lung cancer in those who were GSTM1 null (odds ratio=0·67, 95% CI 0·49–0·91), GSTT1 null (0·63, 0·37–1·07), or both (0·28, 0·11–0·67). No protective effect was seen in people who were both GSTM1 and GSTT1 positive (0·88, 0·65–1·21). Similar protective results were noted for consumption of cabbage and a combination of broccoli and brussels sprouts. These data provide strong evidence for a substantial protective effect of cruciferous vegetable consumption on lung cancer.

Observational studies have provided consistent evidence for a protective role of vegetable consumption against lung cancer, with the evidence being most apparent for green cruciferous vegetables such as broccoli and cabbage.[1] Such vegetables are rich in isothiocyanates , which have been shown in animals to have strong chemopreventative properties against lung cancer.[2] In a review of studies of the effect of cruciferous vegetables, a definite protective effect against any type of cancer could not be identified, in view of the small size of studies and potential for confounding from other dietary sources.[3]

Confounding could, however, be addressed by adoption of a mendelian randomisation approach.[4] Isothiocyanates are thought to be eliminated by glutathione-S-transferase enzymes, most notably GSTM1 and GSTT1.[5,6] Both *GSTM1* and *GSTT1* genes have null alleles with homozygous null genotypes, resulting in no enzyme being produced. Individuals who are homozygous for the inactive form of either or both genes probably have higher isothiocyanate concentrations because of their reduced elimination capacity. Furthermore, and implicit in the mendelian randomisation approach, the roles of *GSTM1* and *GSTT1* genes are likely to be independent of other dietary and lifestyle factors, reducing the possibility of confounding from these sources.

To clarify the role of cruciferous vegetable consumption in the prevention of lung cancer, and their interaction with GST genotypes, large-scale studies are needed. We therefore investigated this relation in a case-control study of 2141 cases and 2168 controls in six countries of central and eastern Europe, a region that has traditionally high rates of cruciferous vegetable consumption. Incident cases and age-sex matched hospital or population controls were recruited from 15 centres in Poland, Slovakia, Czech Republic, Romania, Russia, and Hungary by use of an identical protocol and questionnaire. All participants completed a detailed standardised lifestyle and food frequency questionnaire that had been piloted in all centres before use. The dietary component of the questionnaire listed 23 foods, of which three were cruciferous vegetables (cabbage and a combination of brussels sprouts with broccoli). The questionnaire was repeated for two different periods: the year before interview; and before political and market changes in 1989 (1991 in Russia); a weighted average of the two was calculated on the basis of the age of the individual. A blood sample was also obtained for all individuals, and DNA was extracted. Genotyping for *GSTM1* and *GSTT1* was done with PCR-based techniques (protocol available from the authors, on request).

Neither GSTM1 nor GSTT1 were related to potential confounding factors in the controls, such as country, age, smoking status, education, and dietary variables including cruciferous vegetable consumption (webtable 1). As expected, cruciferous vegetable consumption was related to other dietary variables, such as fruit and other vegetables, and smoking status (webtable 2), although these associations do not detract from the validity of the mendelian randomisation comparison. All interviewees provided written informed consent before their participation in the study. Ethical approval for recruitment of participants and subsequent genotyping was obtained from institutional review boards in all the study centres, and from the International Agency for Research on Cancer (IARC) ethics committee.[7]

An overall protective effect was seen for consumption of cruciferous vegetables at least once a week compared with less than monthly (adjusted odds ratio=0·78, 95% CI 0·64–0·96), which was much the same for both cabbage consumption and for broccoli and brussels sprout consumption (table 1). When stratified by GST

	All cruciferous vegetables			Cabbage			Broccoli and brussel sprouts		
	Cases/controls*	OR (95% CI)†	p	Cases/controls*	OR (95% CI) †‡	p	Cases/controls*	OR (95% CI) †§	p
ALL GST types									
Low	327 / 250	1		367/296	1		1747/1758	1	
Medium	677 / 754	0·77 (0·62–0·95)	0·0156	668/740	0·80 (0·65–0·98)	0·0342	249/261	1·04 (0·84–1·29)	0·7163
High	1137 / 1164	0·78 (0·64–0·96)	0·0188	1106/1132	0·83 (0·69–1·01)	0·0674	145/149	0·87 (0·66–1·14)	0·3118
GSTM1+									
Low	160/ 140	1		177/159	1		842/885	1	
Medium	323/ 368	0·92 (0·68–1·24)	0·5722	319/365	0·92 (0·69–1·24)	0·5996	127/134	1·08 (0·80–1·46)	0·6075
High	560/ 589	0·89 (0·67–1·18)	0·4156	547/573	0·93 (0·70–1·22)	0·5818	74/78	0·94 (0·64–1·39)	0·7695
GSTM1 null									
Low	161/ 103	1		184/128	1		845/801	1	
Medium	330/ 352	0·65 (0·47–0·89)	0·0072	323/342	0·69 (0·51–0·93)	0·0143	113/117	0·98 (0·71–1·35)	0·9122
High	531/ 531	0·67 (0·49–0·91)	0·0092	515/516	0·73 (0·55–0·97)	0·0308	64/68	0·72 (0·47–1·09)	0·1198
GSTT1+									
Low	265/ 211	1		298/247	1		1443/1467	1	
Medium	557/ 614	0·80 (0·63–1·01)	0·0631	546/607	0·81 (0·64–1·01)	0·0630	211/216	1·09 (0·87–1·38)	0·4475
High	953/ 971	0·83 (0·66–1·03)	0·0940	931/942	0·86 (0·70–1·06)	0·1635	121/113	0·97 (0·71–1·32)	0·8317
GSTT1 null									
Low	58/ 37	1		65/46	1		285/270	1	
Medium	115/ 133	0·67 (0·39–1·16)	0·1551	117/127	0·79 (0·47–1·33)	0·3707	33/41	0·80 (0·45–1·44)	0·4593
High	167/ 174	0·63 (0·37–1·07)	0·0849	158/171	0·73 (0·44–1·21)	0·2223	22/33	0·55 (0·27–1·11)	0·0954
GSTM1/GSTT1 +/+									
Low	127/ 114	1		140/129	1		693/753	1	
Medium	263/ 305	0·87 (0·62–1·22)	0·4100	259/306	0·87 (0·63–1·21)	0·4111	106/109	1·15 (0·82–1·59)	0·4191
High	471/ 502	0·88 (0·65–1·21)	0·4391	462/486	0·93 (0·69–1·27)	0·6584	62/59	1·02 (0·67–1·57)	0·9163
GSTM1+/GSTT1 null or GSTM1 null/GSTT1+									
Low	162/ 117	1		186/140	1		828/774	1	
Medium	325/ 339	0·82 (0·60–1·12)	0·2214	316/328	0·81 (0·60–1·10)	0·1756	112/120	0·97 (0·70–1·33)	0·8425
High	516/ 507	0·80 (0·60–1·08)	0·1498	501/495	0·81 (0·61–1·07)	0·1339	63/69	0·81 (0·53–1·24)	0·3362
GSTM1 null/GSTT1 null									
Low	28/ 10	1		31/15	1		147/138	1	
Medium	60/ 69	0·26 (0·10–0·63)	0·0032	62/67	0·37 (0·16–0·84)	0·0169	17/18	0·91 (0·39–2·13)	0·8273
High	87/ 92	0·28 (0·11–0·67)	0·0045	82/89	0·42 (0·19–0·94)	0·0338	11/15	0·35 (0·12–1·01)	0·0529

Null=those with homozygous deletion of GST. +=positive. Low=these vegetables eaten less than once per month; medium=less than once per week; high=at least once per week. *2141 cases, 2168 controls. †Adjusted by age, sex, country, education, and tobacco pack-years. ‡Additional adjustment for intake of broccoli and brussels sprouts. §Additional adjustment for cabbage intake.

Table 1: **Odds ratios for lung cancer by cruciferous vegetable consumption* and GST status**

status, any protective effect of high consumption was restricted to those who were null for *GSTM1* (0·67, 0·49–0·91), *GSTT1* (0·63, 0·37–1·07), or both *GSTM1* and *GSTT1*, (0·28, 0·11–0·67). No protective effect was seen in those who were GSTM1 and T1 positive, with a moderate non-significant protective effect for those who had only one null genotype (0·80, 0·60–1·08). The interaction between GSTM1 null/GSTT1 null versus other GSTM1/GSTT1 groups and cruciferous vegetable consumption was significant (p=0·03). Similar results were observed separately for cabbage and broccoli/brussels sprout consumption, after adjustment for the other, suggesting an independent protective effect of both sources of cruciferous vegetables.

When the population was stratified by smoking history (never *vs* ever), the protective effect in never smokers was largely independent of *GSTM1* and *GSTT1* status, being recorded in those who were positive for both *M1* and *T1* (0·31, 0·14–0·70) and negative for *M1* and *T1* (0·12, 0·01–1·54) (table 2). No interaction was noted for GSTM1/GSTT1 null versus other GSTM1/GSTT1 combinations in never smokers (p=0·70), although there was evidence of interaction in those who had smoked (p=0·05). Since one might expect GST enzyme concentrations to be higher in smokers than in non-smokers because of the presence of tobacco-specific substrates, *GSTM1* and *GSTT1* might not modify the protective effect of cruciferous vegetables in non-smokers.

These results provide strong evidence for our a-priori hypothesis that the protective effect of cruciferous vegetables is most apparent in those who have low values of circulating GST enzymes, because they have null alleles for *GSTM1* and *GSTT1* genes. The results also accord with those of several smaller studies of lung cancer, breast cancer, and colorectal adenomas, which showed a protective effect in *GSTM1* and *GSTT1* null carriers.[2] However, we cannot draw definitive conclusions from these individual studies because of their small sample size (the three previous studies of lung cancer, based in Shanghai, Singapore, and Texas, consisted of a total of 968 cases and 1362 controls).[5,8,9] Taking into consideration the size of the protective effects that have been reported in this and other studies, which are likely to have been substantially diluted by measurement error, these findings raise the prospect of

	Never smokers			Ever smokers		
	Case/control*	OR† (95%CI)	p	Case/control‡	OR§ (95%CI)	p
GSTM1+						
Low	14/36	1		146/104	1	
Medium	28/136	0·52 (0·23–1·18)	0·1165	295/232	1·01 (0·73–1·40)	0·9403
High	39/216	0·36 (0·16–0·79)	0·0107	521/373	1·04 (0·77–1·40)	0·8098
GSTM1 null						
Low	11/27	1		150/76	1	
Medium	25/121	0·57 (0·23–1·42)	0·2258	305/231	0·68 (0·48–0·96)	0·0300
High	39/172	0·47 (0·19–1·14)	0·0951	492/359	0·70 (0·50–0·96)	0·0288
GSTT1+						
Low	22/56	1		243/155	1	
Medium	49/219	0·56 (0·30–1·05)	0·0687	508/395	0·86 (0·66–1·10)	0·2323
High	68/340	0·40 (0·22–0·74)	0·0035	885/631	0·91 (0·72–1·16)	0·4607
GSTT1 null						
Low	3/10	1		55/27	1	
Medium	8/49	0·44 (0·07–2·84)	0·3857	107/84	0·71 (0·39–1·29)	0·2639
High	13/55	0·22 (0·03–1·56)	0·1303	154/119	0·65 (0·37–1·14)	0·1332
M1/T1 +/+						
Low	14/30	1		113/84	1	
Medium	23/110	0·47 (0·20–1·09)	0·0792	240/195	0·98 (0·69–1·41)	0·9337
High	29/182	0·31 (0·14–0·70)	0·0048	442/320	1·07 (0·77–1·50)	0·6806
M1/T1 +/ null or null/+						
Low	8/30	1		154/87	1	
Medium	27/123	0·84 (0·31–2·25)	0·7307	298/216	0·85 (0·61–1·18)	0·3314
High	44/176	0·70 (0·27–1·83)	0·4649	472/331	0·81 (0·59–1·12)	0·2024
M1/T1 null/null						
Low	3/3	1		25/7	1	
Medium	3/22	0·09 (0·01–0·99)	0·0488	57/47	0·30 (0·11–0·81)	0·0176
High	4/22	0·12 (0·01–1·54)	0·1039	83/70	0·31 (0·12–0·82)	0·0176

Null=those with homozygous deletion of GST. Low=less than once per month; medium=less than once per week; high=at least once per week. *164 cases, 739 controls. †In never smokers, model adjusted by age, sex, and education. ‡1977 cases, 1429 controls. §Adjusted by age, sex, country, education, and tobacco pack-years.

Table 2: **Odds ratios for lung cancer by cruciferous vegetable consumption and GSTM1 and GSTT1 status for ever and never smokers**

an important chemopreventive effect against lung cancer for cruciferous vegetables in general, and isothiocyanates in particular.

Contributors

P Brennan, N Szeszenia Dabrowska, L Lissowska, D Zaridze, P Rudnai, E Fabianova, D Mates, V Bencko, L Foretova, V Janout, and P Boffetta jointly designed the study and organised the interviewee recruitment. N Moullan, A Chabrier, R Hung, F Gemignani, J Hall and F Canzian organised biological sample storage, DNA extraction, and genotyping. C C Hsu, R Hung, and P Brennan did the statistical analysis. P Brennan prepared the first draft, and all co-authors contributed to the final draft.

Conflict of interest statement

We declare that we have no conflict of interest

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