# Interaction between smoking, *GSTM1* deletion and colorectal cancer: results from the GSEC study

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Cigarette smoking has inconsistently been associated with an increased risk of colorectal cancer. One of the enzymes responsible for the detoxification of the carcinogenic compounds present in tobacco smoke is glutathione $S$-transferase-μ (GST-μ). The gene that codes for this enzyme is *GSTM1*. In this study, we evaluated the associations and interaction between *GSTM1* deletion, smoking behaviour and the development of colorectal cancer. We performed a pooled analysis within the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC). We selected six studies on colorectal cancer, including 1130 cases and 2519 controls, and restricted our analyses to Caucasians because the number of patients from other races was too limited. In addition we performed a meta-analysis including the studies from the GSEC database and other studies identified on MEDLINE on the same subject. The prevalence of the *GSTM1* null genotype was within the range reported in other studies: 51.8% of the cases had the *GSTM1* null genotype versus 56.6% of the controls. No significant association between the *GSTM1* null genotype and colorectal cancer was found (odds ratio 0.92, 95% confidence interval 0.73–1.14). Our results suggest a possible positive association between lack of the GST-μ enzyme and colorectal cancer for non-smoking women (odds ratio 1.47, 95% confidence interval 0.80–2.70). There was no interaction between the effects of smoking and *GSTM1* genotype on colorectal cancer risk in men and women ($\chi^2 = 0.007$, $p = 0.97$). Our findings do not support an association between the *GSTM1* null genotype and colorectal cancer. In addition, we did not find any modification of the smoking-induced colorectal cancer risk by *GSTM1* genotype

*Keywords:* molecular epidemiology, pooled analysis, metabolic gene polymorphism.

## Introduction

Colorectal cancer is one of the most common types of cancer in the Western World. Its development is influenced by both environmental and genetic factors. One of the factors thought to be an important risk factor for this condition is

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smoking. Tobacco smoke contains polycyclic aromatic hydrocarbons (Butler *et al*. 2001), which have been implicated as a potential cause of colorectal cancer (Welfare *et al*. 1999). An association between tobacco use and colorectal cancer has been identified in several epidemiological studies in the past. However, these associations were not consistent (Slattery *et al*. 1998).

Polycyclic aromatic hydrocarbons and other carcinogens are metabolized by phase I and II enzymes. An important step in the detoxification process is conjugation with glutathione, which is catalysed by glutathione *S*-transferases (GSTs) (Gertig *et al*. 1998, Inoue *et al*. 2001, Loktionov *et al*. 2001). GSTs are involved in the detoxification of many electrophilic compounds (Zhong *et al*. 1993, Slattery *et al*. 1998, Welfare *et al*. 1999, Inoue *et al*. 2001).

Various families of genes encoding different forms of GST in humans have been identified. To date research has been focused on the α (GSTA), μ (GSTM), π (GSTP) and θ (GSTT) classes (Raunio *et al*. 1995, Gertig *et al*. 1998, Welfare *et al*. 1999, Cotton *et al*. 2000). Each class consists of several isoenzymes with a partly overlapping substrate specificity (Saadat and Saadat 2001). Functional polymorphisms have been identified for three of the human genes – *GSTM1*, *GSTT1* and *GSTP1*. For *GSTP1*, two genetic polymorphisms have been shown to be responsible for increased activity of the enzyme for specific substrates. For *GSTM1* and *GSTT1*, the variant is a deletion of the gene. Individuals homozygous for the variant allele do not express the enzyme (Deakin *et al*. 1996, Kiss *et al*. 2000).

The *GSTM1* gene codes for the enzyme GST-μ (Raunio *et al*. 1995, Gertig *et al*. 1998, Cotton *et al*. 2000). It contains three alleles located on chromosome 1p13.3: GSTM1*A, GSTM1*B and a deficient GSTM1*0 (Raunio *et al*. 1995, Zhang *et al*. 1999, Cotton *et al*. 2000). GSTM1*A and GSTM1*B differ by a single amino acid, but no evidence has been found regarding functional differences between them (Cotton *et al*. 2000). The *GSTM1* null genotype has been associated with no enzyme activity and usually with a suggested greater risk for the development of different types of cancer, including colorectal cancer (Deakin *et al*. 1996, Little and Faivre 1999, Kiss *et al*. 2000, Slattery *et al*. 2000, Loktionov *et al*. 2001). Only one study was found that suggested that the *GSTM1* null genotype could protect against colorectal cancer (Lin *et al*. 1998). This study suggested that this genotype protects against the loss of isothiocyanates present in broccoli and some other cruciferous vegetables. However, these results were questioned by other investigators, who suggested that the effect of cruciferous vegetables was independent of the *GSTM1* genotype (Acquavella and Cullen 1999).

In Caucasians, about 50% of the population are known to have the *GSTM1* null genotype (Szarka *et al*. 1995, Gertig *et al*. 1998) and are therefore lacking *GSTM1* activity (Katoh *et al*. 1990, Lin *et al*. 1995).

The *GSTM1* null genotype has been associated with colorectal cancer in several epidemiological studies in the past, but the observed associations are often weak or inconsistent (Lin *et al*. 1995, Szarka *et al*. 1995, Deakin *et al*. 1996, Gertig *et al*. 1998, Little and Faivre 1999, Zhang *et al*. 1999, Cotton *et al*. 2000, Kiss *et al*. 2000, Slattery *et al*. 2000, Loktionov *et al*. 2001). In most of the previous studies, the sample size was relatively small (Katoh *et al*. 1990, Cotton *et al*. 2000). Thus, a

combined analysis of these studies would be very useful in investigating the association between the *GSTM1* null genotype and colorectal cancer and to analyse the interaction between the *GSTM1* null genotype and smoking behaviour in colorectal cancer patients. Therefore, we performed a pooled analysis within the data of the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC) to obtain information concerning the influence of the *GSTM1* null genotype on colorectal cancer risk. In addition, we assessed the interaction between this genotype and smoking behaviour in a subset of the data used for the pooled analysis.

## Materials and methods

*Study population*

Study participants were from the GSEC. This study was started as a collaborative project to collect and analyse information on polymorphisms in a number of metabolizing genes and environmental factors from published and unpublished case-control studies all over the world. Investigators who had performed a case-control study on genetic polymorphisms were contacted and asked to participate in the project by sending their original data. The design of this study is described in detail elsewhere (Taioli 1999).

Eight studies were identified from the GSEC database in which information on colorectal cancer and *GSTM1* genotype was presented (Chenevix-Trench *et al.* 1995, Deakin *et al.* 1996, Dolzan *et al.* unpublished data, Seidegard *et al.* unpublished data, Taioli and Garte unpublished data, Lechner *et al.* unpublished data, Golka *et al.* unpublished data, Kristensen *et al.* unpublished data). One of these was a case-only study, with no information on smoking, and was therefore excluded (Chenevix-Trench *et al.* 1995). The number of patients with colorectal cancer in the studies among non-Caucasians was too limited, so we decided to restrict our analyses to Caucasians and one study among non-Caucasians was therefore excluded (Taioli and Garte unpublished data). Controls aged < 20 years were excluded from the analyses because they were not comparable to the colorectal cancer cases. For the analysis that included smoking behaviour, four more studies from the GSEC database were excluded due to lack of information on smoking status for either the cases or the controls. Using a search on MEDLINE, three other studies on colorectal cancer, smoking and *GSTM1* genotype were identified (Lin *et al.* 1995, Gertig *et al.* 1998, Slattery *et al.* 1998). Only one of the studies not included in the GSEC database presented information on *GSTM1* deletion stratified by smoking status (Gertig *et al.* 1998); the information from this study was added to the information from the GSEC studies and a meta-analysis was performed (Table 1).

Table 1. Number of cases and controls in studies used in the analysis according to smoking status.

| Study | GSTM1 | Non-smokers | | Smokers | |
|---|---|---|---|---|---|
| | | Cases | Controls | Cases | Controls |
| Lechner *et al.* Unpublished data[a] | Present | 19 | 112 | 19 | 43 |
| | Null | 28 | 187 | 14 | 59 |
| Golka *et al.* Unpublished data | Present | 38 | 38 | 63 | 59 |
| | Null | 35 | 35 | 56 | 54 |
| Gertig *et al.* 1998 (not in GSEC database) | Present | 36 | 40 | 53 | 58 |
| | Null | 41 | 40 | 61 | 62 |

Two additional studies (Slattery *et al.* 1998, Lin *et al.* 1998) did not include data on smoking and *GSTM1* and are therefore excluded.

[a] Gil J. P. 2000, Polymorfismo dos genes *CYP2D6*, *GSTM1*, *GSTP1* e *NAT2* na população Portuguesa. Pesquisa de marcadores de susceptibilidade a patologias oncológicas. [Polymorphisms in *CYP2D6*, *GSTM1*, *GSTP1* and *NAT2* in the Portuguese population. Markers of susceptibility to cancer.] PhD in Molecular Biology, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa. Portugal. PhD grant PRAXIS/BD/3357/94, FCT, Portugal, PhD thesis.

*Data collection*

Information about the *GSTM1* genotype was available for all studies. Information about other variables such as age and sex was not available for all participants. Two studies did not report any information about the age of the cases and the controls (Dolzan *et al*. unpublished data, Kristensen *et al*. unpublished data). Smoking status was reported in two of the selected studies from the GSEC database, and was categorized as never versus ever smokers (Lechner *et al*. unpublished data, Golka *et al*. unpublished data).

Every author that sent data to be included in the GSEC database also sent a list with additional information on how data was collected. Information on demographic characteristics and smoking habits had been gathered using questionnaires in each of the original studies. Information about the *GSTM1* genotype was obtained, without exceptions, using a standard polymerase chain reaction.

*Statistical methods*

Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were computed by unconditional logistic regression models (Rothman 1986) using individual information for cases and controls from the GSEC database. ORs were adjusted for sex and age. Further adjustment for the type of controls used in the different studies (healthy and hospital) did not alter the results. Individuals with a missing value on one of these variables were excluded from the analyses that included that variable. Stratified logistic regression analyses were performed for never and ever smokers and for men and women separately. In addition, an interaction analysis was performed to assess the interaction between smoking and the *GSTM1* genotype in determining colon cancer risk.

Meta-ORs were calculated based on random effects models. All statistical analyses were performed using SAS software release 8.01.

## Results

Table 1 summarizes the studies that were included in the meta-analysis performed on *GSTM1* genotype and colorectal cancer stratified for smoking. As shown, only three studies had information on both *GSTM1* genotype and smoking status.

The studies selected from the GSEC database included 1130 cases and 2519 controls. The *GSTM1* genotype was not available for all the participants. The actual numbers used for the analyses are presented in Table 2, which includes details on the number of cases and controls in the selected studies with a known *GSTM1* genotype, the mean age of the participants, the type of controls used in the study, the number of persons with a *GSTM1* null genotype and the number of smokers. The *GSTM1* genotype was available for 874 cases and 2125 controls. For two studies no information on age was available; therefore 575 cases and 1935 controls had information on both age and *GSTM1* genotype. Smoking status was completely absent in two studies and too scarce to use in the calculations in two other studies. One study was performed using a case-only design, and was excluded from the analysis.

The *GSTM1* null genotype was present in 51.8% of the cases and in 56.6% of the controls.

The association between the *GSTM1* null genotype and colorectal cancer for the whole population and for men and women separately is presented in Table 3. The crude OR suggested a protective effect for the *GSTM1* null genotype, but this effect disappeared when the analysis was adjusted for age and sex. No differences according to sex were observed in the association between colorectal cancer and *GSTM1* null genotype in terms of either the unadjusted or the age-adjusted OR.

In addition, we performed analyses stratified for smoking in order to assess whether the influence of the *GSTM1* null genotype on colorectal cancer differs for

Table 2.   Description of the studies included in the pooled analysis.

| Study | No. with known genotype | Cases Age (mean± SD; years) | GSTM1 null | Ever smokers | No. with known genotype | Source | Controls Age (mean± SD; years) | GSTM1 null | Ever smokers |
|---|---|---|---|---|---|---|---|---|---|
| *Case-control studies* | | | | | | | | | |
| Dolzan *et al.* unpublished data | 58 | – | 29 (50.0%) | – | 102 | Healthy | – | 53 (48.0%) | – |
| Deakin *et al.* 1996 | 213 | 66.8±11.3 | 110 (51.6%) | – | 482 | Hospital | 59.9±17.8 | 281 (58.3%) | 64 (46.0%) |
| Seidgard *et al.* unpublished data | 95 | 66.7±11.8 | 53 (56.0%) | 3 (60.0%) | 795 | Healthy | 31.9±11.4 | 455 (57.2%) | – |
| Lechner *et al.* unpublished data | 114 | 63.3±11.1 | 59 (51.7%) | 28 (37.3%) | 467 | Hospital | 51.8±17.8 | 271 (58.0%) | 88 (24.0%) |
| Golka *et al.* unpublished data | 153 | 66.9±10.0 | 81 (52.9%) | 89 (59.3%) | 191 | Hospital | 62.9±10.4 | 91 (47.6%) | 113 (60.7%) |
| Kristensen *et al.* unpublished data | 241 | – | 121 (50.2%) | – | 88 | Healthy | – | 52 (59.1%) | – |
| *Case-only studies* | | | | | | | | | |
| Chenevix-Trench *et al.* 1995 | 115 | 68.0±13.0 | 58 (50.4%) | – | | | | | |

Table 3. Association between *GSTM1* null genotype and colorectal cancer.

|  |  | Cases | Controls | OR | 95% CI |
|---|---|---|---|---|---|
| Total population | Unadjusted | 874 (29.1%) | 2125 (70.8%) | 0.82 | 0.70–0.96 |
|  | Adjusted[a] | 552 (24.8%) | 1676 (75.2%) | 0.92 | 0.73–1.14 |
| Men | Unadjusted | 321 (19.7%) | 1304 (80.3%) | 0.84 | 0.65–1.07 |
|  | Adjusted[b] | 307 (21.2%) | 1139 (78.8%) | 0.90 | 0.67–1.22 |
| Women | Unadjusted | 250 (26.9%) | 680 (73.1%) | 0.90 | 0.67–1.20 |
|  | Adjusted[b] | 245 (31.3%) | 537 (68.7%) | 0.93 | 0.68–1.29 |

[a]OR adjusted for age, sex and study.
[b]OR adjusted for age and study.

smokers compared with non-smokers. The meta-analysis and pooled analysis showed no significant association between colorectal cancer and the *GSTM1* null genotype for never smokers as well as ever smokers (Table 4). In the pooled analysis, we were able to stratify the data for both smoking status and sex. Although female never smokers showed an increased risk of colorectal cancer with *GSTM1* deletion, the association was not statistically significant.

Table 5 shows the analysis of the interaction between smoking and *GSTM1* null genotype. Our results did not suggest a modification of the carcinogenic effect of smoking according to the *GSTM1* genotype. Among ever smokers, the risk of colorectal cancer was similar between subjects carrying the *GSTM1* functional gene and those carrying the complete deletion. The test for interaction in the logistic model was not significant ($\chi^2 = 0.007$, $p > 0.99$). Similar results were obtained using the meta-analysis data set.

## Discussion

An increasing number of studies indicate that polymorphisms in genes that code for metabolic enzymes are involved in the development of several different types of cancer because they alter the activity of the enzyme. For colorectal cancer, one of the polymorphisms that has received considerable attention is *GSTM1* polymorphism. The general hypothesis is that people with the *GSTM1* null genotype, who lack enzyme activity, have a higher risk of developing colorectal cancer.

The aim of the present study was to analyse the results of previous studies on the association between the *GSTM1* null genotype and colorectal cancer. We were especially interested in the association between the *GSTM1* null genotype and smoking in colorectal cancer, since the polycyclic aromatic hydrocarbons that are present in tobacco smoke are known to be metabolized by GSTM1.

Several studies have previously been conducted to assess the association between the *GSTM1* null genotype and colorectal cancer. However, these results are not consistent. Only one study showed an increased risk of colorectal cancer for the *GSTM1* null genotype that was statistically significant (Zhong *et al.* 1993); most studies showed a small increased risk that was not statistically significant. Recently, a HuGE review was published on colorectal cancer and GST polymorphisms (Cotton *et al.* 2000), which concluded that it is not clear whether

Table 4. Association between *GSTM1* null genotype and colorectal cancer according to smoking status.

| | | Never smokers | | | | Ever smokers | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cases | Controls | OR | 95% CI | Cases | Controls | OR | 95% CI |
| Meta-analysis | | 197 (30.4%) | 452 (69.6%) | 0.81 | 0.58−1.13 | 266 (44.3%) | 335 (55.7%) | 0.89 | 0.64−1.22 |
| GSEC data | Unadjusted | 110 (20.7%) | 420 (79.3%) | 0.85 | 0.56−1.31 | 120 (31.2%) | 265 (68.8%) | 0.89 | 0.58−1.37 |
| | Adjusted[a] | 108 (23.8%) | 345 (76.2%) | 1.08 | 0.68−1.72 | 115 (36.4%) | 201 (63.6%) | 1.09 | 0.67−1.78 |
| Men | Unadjusted | 47 (25.7%) | 136 (74.3%) | 0.61 | 0.31−1.18 | 99 (38.4%) | 159 (61.6%) | 0.94 | 0.57−1.56 |
| | Adjusted[b] | 47 (28.0%) | 121 (72.0%) | 0.69 | 0.34−1.40 | 96 (42.8%) | 128 (57.2%) | 1.08 | 0.63−1.87 |
| Women | Unadjusted | 63 (18.1%) | 284 (81.9%) | 1.05 | 0.60−1.82 | 20 (16.0%) | 105 (84.0%) | 0.87 | 0.34−2.28 |
| | Adjusted[b] | 61 (21.4%) | 224 (78.6%) | 1.47 | 0.80−2.70 | 19 (20.6%) | 73 (79.4%) | 1.11 | 0.37−3.35 |

[a]OR adjusted for age, sex and study.
[b]OR adjusted for age and study.

Table 5.   Interaction analysis of *GSTM1* genotype and smoking status.

| Smoking status | GSTM1 | GSEC data | | | | Meta-analysis data | | |
|---|---|---|---|---|---|---|---|---|
| | | Cases | Controls | OR (95% CI) | Adjusted OR (95% CI)[a] | Cases | Controls | OR (95% CI) |
| Never | Present | 48 | 150 | 1.00 (reference) | 1.00 (reference) | 84 | 190 | 1.00 (reference) |
| Never | Null | 60 | 195 | 0.96 (0.62–1.49) | 1.06 (0.67–1.69) | 101 | 235 | 0.97 (0.69–1.38) |
| Ever | Present | 57 | 102 | 1.75 (1.10–2.76) | 1.79 (1.07–2.99) | 110 | 160 | 1.56 (1.09–2.21) |
| Ever | Null | 58 | 99 | 1.83 (1.16–2.90) | 1.95 (1.17–3.26) | 119 | 161 | 1.67 (1.18–2.37) |

[a]OR adjusted for age, sex, and study.
Test for interaction: $\chi^2 = 0.007$, $p > 0.99$, d.f. $= 1$.

*GSTM1* genotype is associated with colorectal cancer and that more research is needed to confirm previous results.

The prevalence of the *GSTM1* null genotype in the cases in our study is comparable to that in the controls and is in good accordance with previously published data (Garte *et al.* 2001). The results from our study suggested a protective effect for the null genotype for the total population as well as for men and women separately in the unadjusted analyses. However, this effect completely disappeared when the analyses were adjusted for age and sex. In the adjusted analysis, no significant association could be found between the *GSTM1* null genotype and colorectal cancer for the total population. For men and women separately, again no effect of *GSTM1* deletion on the colorectal cancer risk was observed.

To our knowledge, three other studies on the *GSTM1* null genotype and smoking in colorectal cancer were performed that were not included in the GSEC database (Lin *et al.* 1995, Gertig *et al.* 1998, Slattery *et al.* 1998). From these studies only the study of Lin *et al.* (1995) suggested a statistically significant increase in colorectal cancer risk for current smokers who had the *GSTM1* null genotype. The results of the other studies did not suggest an interaction between smoking and the *GSTM1* null genotype in colorectal cancer.

A meta-analysis was performed including the information from one of the three studies that stratified the data for both smoking and *GSTM1* together with the selected studies from the GSEC database. The addition of this information did not alter the results. The other two studies could not be included in the meta-analysis because no information on the absolute numbers of smokers among cases and controls was available (Lin *et al.* 1998, Slattery *et al.* 1998). One of these studies suggested a positive association between the *GSTM1* null genotype and colorectal cancer for current smokers (OR 1.73, 95% CI 1.03–2.90) (Lin *et al.* 1995). The other study did not show any association between the *GSTM1* genotype and ever or never smokers (Slattery *et al.* 1998). It is believed that including these studies would not have altered our conclusion.

In our study, we did not find a difference between smokers and non-smokers in the association between *GSTM1* and colorectal cancer. When we performed the analyses stratified for men and women, our study suggest a small increase in colorectal cancer risk for women that have the *GSTM1* null genotype and are non-smokers. However, statistical significance was not reached for these results. As far as we know, no other study has ever reported this difference between smoking and non-smoking women with the *GSTM1* null genotype. Because of this, it is possible that this finding is due to chance.

We also performed an interaction analysis. To our knowledge, this is the first formal interaction analysis of *GSTM1* genotype and smoking in colorectal cancer. The results of this analysis suggest that the *GSTM1* null genotype slightly increases the carcinogenic effect of smoking. However, this result was not statistically significant. From this, we can conclude that there is no interaction between the *GSTM1* genotype and smoking, and that the *GSTM1* genotype does not modify the smoking-induced colorectal cancer risk.

In the past, several studies have been conducted to investigate the association between colorectal cancer and cigarette smoking. Results from these studies were not consistent. Although some studies suggested a positive association between colorectal cancer and smoking behaviour (Wu and Henderson 1995, Slattery *et al.* 2000, Giovannucci 2001), other studies have not confirmed these results (Sandler *et al.* 1993). Because of the important role played by GSTs in the detoxification of smoking-related carcinogens, they are frequently studied in cancers that are more consistently associated with cigarette smoking, such as lung cancer.

For colorectal cancer, however, studies performed on smoking and the *GSTM1* null genotype are not very frequent, and have almost all been included in our analysis. Only one of the studies showed a statistical significant result (Lin *et al.* 1995).

Our findings do not support an interaction between the *GSTM1* null genotype and smoking for colorectal cancer. However, our results show an increased risk for colorectal cancer in women after stratification for smoking status. This increase was greatest for women who did not smoke. This finding is not supported by other studies in which, after stratification for sex, women with the null genotype were observed to have a smaller risk of developing colorectal cancer (Slattery *et al.* 1998).

There are several limitations to our study. Although it was a pooled analyses and therefore larger than some previous studies, little information was available on the smoking status of the cases. Consequently, the number of persons in the analyses that included smoking data was not very large. In addition, some of the stratified analyses performed in this study produced cells with a very small number of subjects, thus reducing the significance of some of the findings. For some variables that are known to influence the risk on colorectal cancer, such as family history, very little information was available. Therefore, these variables could not be included in the model.

Usually when pooled analyses are performed, differences between the individual studies can cause bias. In our study this is not very likely because the laboratory methods used in the selected studies were standard polymerase chain reactions. In addition, the percentage of *GSTM1* deletion among controls was similar to that reported in the literature (Garte *et al.* 2001). In the two studies used for the analyses related to smoking, the methods used to assess smoking status were similar in that they were both questionnaires. However, the percentages of smokers among cases and controls were very different among the studies. This can probably be explained by different smoking habits in the countries the subjects come from or by differences in data collection.

Another aspect to consider is the use of unpublished data in the pooled analyses. One of the reasons that data remains unpublished is that it yielded negative results. This could imply that negative studies were over-represented in our pooled analyses. However, inclusion of the study of Gertig *et al.* (1998) in the meta-analyses did not alter our conclusions. Therefore it is not likely that our conclusions were influenced by the fact that unpublished data were used.

In summary, we can conclude that there was no apparent relationship between *GSTM1* genotype and colorectal cancer, and that it is likely that *GSTM1* genotype

does not alter the smoking-induced colorectal cancer risk. However, larger studies including information on several metabolic gene polymorphisms, detailed smoking history and other epidemiological information are needed.

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