NAT2, Meat Consumption and Colorectal Cancer Incidence: An Ecological Study of 27 Countries

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ABSTRACT

Aim: The polymorphic gene NAT2 is a major determinant of N-acetyltransferase activity and, thus, may be responsible for one's ability to bioactivate heterocyclic amines, a class of procarcinogens in cooked meat. Marked geographic variations in phenotype distribution have been observed for NAT2. The present study re-examines the positive international correlation reported for meat intake and colorectal cancer (CRC) incidence and evaluates the potential modifying effects of NAT2 phenotype and other lifestyle factors on this correlation.

Design: This ecological study was based on international data for 1990-2000 CRC incidence, per capita meat consumption, prevalence of fast/intermediate NAT2 phenotype, smoking, and other dietary factors for 27 countries in 5 continents. Regression models were fit and partial correlation coefficients were computed for men and women separately.

Results: For males, inclusion of fast/intermediate NAT2 phenotype with meat consumption improved the fit of the regression model for CRC incidence (R^2 = 0.77, compared to R^2 = 0.70 for meat alone; p for difference in model fit: 0.04). Similar results were obtained for CRC incidence in women (R^2 = 0.76 compared to R^2 = 0.69; p= 0.01). For both sexes, both meat consumption and NAT2 phenotype were associated with CRC

incidence ($p<0.001$ and $p<0.05$, respectively). Smoking prevalence and alcohol consumption had no effect on the models. The partial correlation between NAT2 and CRC incidence was 0.51 in males and 0.47 in females after controlling for meat consumption. No interaction was observed between meat consumption and NAT2 in either sex.

Conclusion: Fast/intermediate NAT2 phenotype is positively correlated with CRC incidence across populations and the effect seems to be additive to that of meat intake. Therefore, in addition to meat intake, some proportion of the international variability in CRC incidence may be attributable to genetic susceptibility determined by NAT2 genotype.

Key Words: Colorectal Cancer, Ecological Studies, Meat, NAT2

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in most developed countries, whereas less than one third of the world's CRC cases occur in developing countries (Parkin et al., 1993). One possible risk factor that is more common in developed countries is a high meat diet. Although the association of meat intake with CRC risk has been relatively consistent in studies conducted in the US, this relationship remains controversial, as it has not been observed as consistently in other parts of the world, particularly in Europe (Hill MJ, 1999). Meta-analyses of the published literature on meat consumption and CRC showed a weak direct association for prospective studies and no significant association for case-control studies (Sandhu et al., 2001, Norat et al., 2002). In addition to methodological differences across studies, the inconsistent results may also be explained by differences in meat preparation methods and genetic background across populations (Kampman et al., 1999).

Development of CRC may be initiated or enhanced by the presence of heterocyclic amines, procarcinogens formed when meat is cooked at high temperatures and metabolized through a genetically modulated process. These compounds need to be bioactivated first by CYP1A2 in the liver, then by N-acetyltransferase enzymes (particularly, NAT2) in the liver and colon, before they can damage DNA. A number of common sequence variants (polymorphisms) have been described in the *NAT2* gene that confer a "slow acetylator" phenotype and vary markedly in frequency across populations. The "rapid acetylator" phenotype has been associated with CRC risk, with a relatively weak main effect (OR<2; Illet et al., 1987, Lang et al., 1986, Chen et al., 1998). NAT2 phenotype can be assessed by dosing subjects with a substrate (such as isoniazid,

sulfamethazine or coffee) and measuring urinary metabolites to characterize the subject as “slow” or “rapid” acetylator. Genotyping of a few common slow alleles can also be used to infer the phenotype with only limited misclassification (Le Marchand et al., 1996; Sinha et al., 1994; Deitz AC et al 2004). A recent review on NAT2 polymorphisms and colorectal cancer (Brockton et al., 2000) reported inconsistent results with only one study showing a positive association (Gil et al., 1988), and five studies finding no association. However, those studies that attempted to consider both the NAT2 variants and exposure to meat carcinogens showed a stronger association with CRC (Brockton et al.., 2000). For example, a small case-control study on acetylator status and CRC found no main effect association, but rapid and intermediate acetylators who frequently consumed fried meat were at a significantly increased risk of CRC (Welfare et al., 1997). Similarly, a small Australian case-control study found an association between meat consumption and CRC risk among rapid acetylators only (Roberts-Thomson et al.). The prospective Physician’s Health Study also found a greater increase (OR=1.5) in CRC risk for high vs. low meat consumption among rapid acetylators than among slow acetylators (Chen et al., 1998).

Historically, ecological studies were among the first to suggest an association between meat consumption and CRC incidence (Doll and Peto, 1981). A recent study on the correlation of olive oil and diet with CRC incidence among 28 countries, showed that the models including fish and olive oil, in addition to meat, better predicted CRC incidence, while the inclusion of total fat or animal fat did not improve the fit of the model (Stoneham et al., 2000). We re-examined the correlation of diet and CRC incidence to

explore whether the inclusion of a genetic component, NAT2 phenotype, would help to further explain the international variability in CRC incidence.

MATERIALS AND METHODS

Data sources

The data were extracted from 3 international sources. Age standardized CRC incidence rates for 27 countries were obtained from the GLOBOCAN database (International Agency for Research on Cancer, 1998), which is based on the data from Volume VII of Cancer Incidence in Five Continents (Parkin et al., 1997). These incidence rates primarily cover the period from 1988 to 1992 (Parkin et al., 2001). The data on worldwide per capita meat consumption was obtained from the Food Balance Sheet for 1970, 1980, 1990 and 2000 in the FAOSTAT 2004 database (Food and Agriculture Organization of the United Nations). Fish, animal fat, vegetable, fruit and alcohol consumption data were obtained from the same source, for the year 1990. Smoking per capita and cigarette consumption data for the year 1990 were obtained from Tobacco Control Country Profiles (World Health Organization, 2003). Population-specific NAT2 phenotype/genotype data were extracted from a recent HuGe review on NAT polymorphisms and CRC (Brockton et al., 2000)

Statistical methods

Meat consumption was plotted to examine yearly and inter-country differences in intake. Coefficients of variation were calculated to assess the fluctuation of the reported intake over the years of available data. Consumption of meat and other dietary factors was

expressed per capita. NAT2 prevalence was expressed as a percentage. CRC incidence rates were average annual rates, age-standardized to the world standard population and given per 100,000 individuals. The correlation between male and female CRC incidence was examined and found to be high (r=0.97). Nevertheless, all analyses were looked at separately for males and females to allow for possible gender-related disparities in the relationships of interest. Statistical analyses were completed using SAS 9.1 (SAS, Cary, NC, USA).

The relationship between meat consumption, other dietary factors, smoking, NAT2 phenotype and CRC incidence was first examined by calculating simple (Pearson) correlation coefficients. Partial correlation coefficients were then calculated to determine the strength of the relationship between each individual variable and CRC incidence controlled for each of the other variables separately. The correlations for the explanatory variables were examined and only meat and animal fat consumption were highly correlated.

The relationship between CRC incidence and the potential explanatory factors was further explored using linear regression modeling. A stepwise procedure was implemented to identify important predictors in the models for men and women. The inclusion of variables in a model was limited to those factors that improved the fit of the model based on a significance level of 0.10. The criterion for a variable to stay in a model was set at 0.15. Hence, each time a variable was added to a model, the significance of all of the terms in the model would be assessed and any variable not

reaching a significance of 0.15 would be discarded. All explanatory variables were allowed to enter the models and no variables were forced into the models. Variance inflation factors were examined for the models including both meat and animal fat and there was no strong suggestion of collinearity. The fit of different models was assessed by determining the percentage of variation (inter-country variability) explained by each model.

RESULTS

The increase in meat consumption from 1970 to 2000 for each of the 27 countries is shown in Fig.1. For these countries, the correlations between meat consumption values for 1970, 1980 and 1990 were very high. For example, the correlation coefficient between meat consumption in 1980 and 1990 was 0.92. Therefore, although we used 1990 meat consumption data in our models, the other datasets would have yielded similar results.

The analysis of unadjusted (Pearson's) correlations between any single variable (dietary or NAT2) and CRC showed the strongest correlation for meat intake (r=0.84 for males and 0.83 for females) followed by animal fat and a much smaller positive correlation for fish intake (r=0.40 for males and 0.31 for females) (Table 1). No correlation was observed between NAT2 and CRC (r=0.08 for males and 0.07 for females). Interestingly, NAT2 had the highest partial correlation after controlling for meat intake

(r=0.51 for males and 0.47 for female), followed by fish for males (r=0.48) and fish and animal fat (r=0.31 for both) for females Animal fat was highly correlated with CRC incidence only in the absence of meat (r=0.66 for males and 0.71 for females).Controlling for meat intake diminished the correlation to non-significance, resulting in partial correlation coefficients (PCC) of 0.18 in males and 0.31 in females. Controlling for animal fat, on the other hand, only reduced the PCC for meat and CRC incidence from 0.84 to 0.69 in males and from 0.83 to 0.66 in females. This correlation still remained highly statistically significant ($p<0.0001$) in both sexes.

The stepwise procedure identified first meat, then NAT2, followed by vegetables and finally fish as potentially important predictor variables for the male model, and meat, NAT2 and vegetables for the female model (Table 2). Vegetables played protective role in both models (negative regression coefficient), while fish did not significantly contribute to the female model (Table 2).

The inter-country variability explained by each model was 86% for males and 81% for females (R^2 value in step 4 for male and step 3 for female model, Table 2). The addition of any other factors, such as alcohol consumption or smoking prevalence, had no effect on the models (data for alcohol shown in Table 1). The backward elimination procedure resulted in the same final models.

The inclusion of fruits and vegetables in the models testing the potential confounding of these two variables showed only modest protective effect with PCC of -0.01 for fruits and -0.05 for vegetables, only latter being statistically significant ($P<0.05$) (Table 2).

Fig.2 A-D shows the agreement between the actual CRC rates and those obtained by selected models. Figure 2A for males and 2C for females show the fit of the model for

meat alone. For comparison, Figure 2B and 2D show the fit of the model including meat, NAT2, vegetables and fish (male) or all these except fish (female).

The male model that included only meat (Figure 2A) vastly under-predicted CRC rates for Japan and to a lesser extent for China and New Zealand. It over-predicted rates for Mali, Turkey and Saudi Arabia, compared to the multi-variable model (Figure 2C) where the predicted rates for these countries were more similar to the observed rates. The rates for Bangladesh, Gabon and the United Arab Emirates (UAE) remained over-predicted even in the multi-variable model, while those for Malaysia remained under-predicted.

For females, the model including only meat (Figure 2B) under-predicted CRC rates for Japan, Sweden and New Zealand, and over-predicted rates for Mali, Gabon, Saudi Arabia and the UAE. The CRC rates for all these countries were more similar to the observed rates in the multi-variable model (Figure 2D), except for the UAE. The rates for Nicaragua and Malaysia remained under-predicted even with the multi-variable model.

Discussion

In this ecological study of meat intake and CRC incidence, we showed that the genetic susceptibility examined by proportion of fast/intermediate NAT2 phenotype in different countries has additive effect to meat intake and that some proportion of variability in CRC incidence worldwide may be attributable to NAT2 phenotype.

The direct correlations between meat and animal fat intake and CRC incidence have consistently been observed in ecological studies. Although fish intake has not usually been found to be associated with CRC in analytical studies (COMA reference, 1998), it is possible that fish consumption may increase CRC risk due to the increased intake of animal protein and fat (Stoneham et al., 2000). Dietary factors which may have a

protective effect on CRC, such as vegetables and fruits, were not expected to increase the correlation between meat intake, NAT2 phenotype and CRC incidence but were included in our models due to their potential confounding effect. Indeed, both foods showed slight protective effect.

In addition to lifestyle factors, genetic background plays an important role in cancer development. Genetically determined differences in production of endogenous carcinogens, metabolism of carcinogens, repair of DNA damage, cell proliferation and defense mechanisms contribute to heterogeneity in the susceptibility to cancer development. However, the relative importance of both environmental and genetic factors varies between individuals.

Our study focused on the polymorphic gene NAT2, due to its involvement in the bioactivation of heterocyclic amines, a class of carcinogens derived from the pyrolysis of creatine and creatinine in meat or fish cooked at high temperatures. The acetylator phenotype, assessed by NAT2 genotyping or drug metabolism methods, is usually classified as “fast” (including rapid and intermediate acetylators) or “slow” including only slow activity phenotype. Although the fast acetylator phenotype has been reported to be linked to an increased CRC risk (Roberts-Thompson, 1994, Ilett et al., 1987), this association has not been consistently observed (Ladero et al., 1991, Lang et al., 1997).

Our results support the hypothesis that NAT2 acetylator status modulates the risk of CRC incidence associated with high meat consumption. NAT2 showed no Pearson’s correlation with CRC incidence for males or females, but a relatively high partial correlation in models including meat. The contribution of other dietary factors, such as fish and animal fat, to increased CRC risk is only modest. A diet high in animal fat

represents an independent risk factor and has been linked to increased risk of CRC in many epidemiologic studies summarized in a recent review (Campos et al., 2005).

Our results are consistent with previous studies of populations in which increased meat consumption and dramatic changes in CRC incidence have occurred, such as in migrants from Japan to the US, or, more recently, Japanese in Japan. The former studies suggested a combined influence of genetic (rapid NAT2 and CYP1A2 phenotype) and dietary factors (well-done red meat) (Le Marchand et al., 1999). Indeed, the amount of inter-country variability explained by our models is very high (86% for male and 81% for female). This study focused on dietary factors that are potential sources of heterocyclic amines (meat and fish) and examined animal fat because it is often a component of these two factors and is highly correlated with meat intake. Fruit and vegetable intake were assessed as important confounders and vegetable intake was found to be significantly negatively associated with CRC incidence for men and women. This is consistent to the results reported by Stoneham et al. (2000) in their study of diet, olive oil and CRC risk. The limitations of this study are primarily due to its ecologic nature. Findings on group (country) attributes do not necessarily reflect the status of CRC risk on the individual level. Therefore it is not possible to draw direct connections between individual dietary, lifestyle and genetic properties and CRC risk. Marked geographic variability in distribution of fats, intermediate NAT2 phenotype as well as dietary habits allowed for testing the possible associations on the country level, which open the way for more rigorous epidemiologic studies to explore these associations at the individual level.

In conclusion, these data provide additional support for the hypothesis that a Western diet, a hallmark of which is high meat consumption, combined with genetic susceptibility (rapid NAT2 phenotype) lead to increased CRC incidence.

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