CYP1A1, *GSTM1*, and *GSTT1* Polymorphisms, Smoking, and Lung Cancer Risk in a Pooled Analysis among Asian Populations

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Abstract

To evaluate the roles of *CYP1A1* polymorphisms [*Ile*^{462}*Val* and T^{6235}*C (MspI)*] and deletion of *GSTM1* and *GSTT1* in lung cancer development in Asian populations, a pooled analysis was conducted on 13 existing studies included in Genetic Susceptibility to Environmental Carcinogenesis database. This pooled analysis included 1,971 cases and 2,130 controls. Lung cancer risk was estimated as odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression model adjusting for age, sex, and pack-year. The *CYP1A1* ^{6235}*C* variant was associated with squamous cell lung cancer (TC versus *TT*: OR, 1.42; 95% CI, 0.96-2.09; *CC* versus *TT*: OR, 1.97; 95% CI, 1.26-3.07; P_{trend} = 0.003). In haplotype analysis, ^{462}*Val*-^{6235}*T* and *Ile-C* haplotypes were associated with lung cancer risk with reference to the *Ile-T* haplotype (OR, 3.41; 95% CI, 1.78-6.53 and OR, 1.39; 95% CI, 1.12-1.71, respectively). The *GSTM1*-null genotype increased squamous cell lung cancer risk (OR, 1.36; 95% CI, 1.05-1.77). When the interaction was evaluated with smoking, increasing trend of lung cancer risk as pack-year increased was stronger among those with the *CYP1A1* 6235 *TC/CC* genotype compared with those with *TT* genotype ($P_{interaction}$ = 0.001) and with the *GSTM1*-null genotype compared with the present type ($P_{interaction}$ = 0.08, when no genotype effect with no exposure was assumed). These results suggest that genetic polymorphisms in *CYP1A1* and *GSTM1* are associated with lung cancer risk in Asian populations. However, further investigation is warranted considering the relatively small sample size when subgroup analyses were done and the lack of environmental exposure data other than smoking. (Cancer Epidemiol Biomarkers Prev 2008;17(5):1120–6)

Introduction

Lung cancer mortality has increased rapidly during recent years in Asian countries. Cigarette smoking is the strongest established risk factor for lung cancer, but genetically determined variations in metabolism of tobacco-derived carcinogens may affect individual susceptibility to lung cancer. Cigarette smoke contains a variety of carcinogens, such as polycyclic aromatic hydrocarbons, *N*-nitrosoamines, and aromatic heterocyclic amines (1). These carcinogens undergo biotransformation by several enzymatic pathways, including P450s (CYP), glutathione *S*-transferase (GST), and *N*-acetyltransferase.

CYP1A1 plays an important role in the metabolism of polycyclic aromatic hydrocarbons, including benzo(*a*)pyrene, as a phase I enzyme and two variants (i.e., *Ile*^{462}*Val* and T^{6235}C), which are potentially functional (2-4), have been evaluated as susceptibility factors for lung cancer by a number of investigators. An increased risk of lung cancer has been observed with the ^{6235}C variant among smokers (5) and with ^{462}*Val* among nonsmokers (6) in

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previous pooled analyses using the Genetic Susceptibility to Environmental Carcinogenesis (GSEC) database, whereas a separate meta-analysis did not find a significant association with lung cancer risk (7).

GSTM1 catalyzes reactive electrophilic intermediates derived from cigarette smoking, such as benzo(*a*)pyrene-7,8-diol-9,10-epoxides (BPDE), to less reactive and more easily excreted glutathione conjugates (8). Deletion of *GSTM1* has most widely been evaluated for the association with lung cancer risk and a significant association was found in several studies. Although three meta-analyses concluded that the *GSTM1*-null genotype is associated with an increased lung cancer risk (9-11), a GSEC pooled analysis indicated that there is no strong evidence for increased risk of lung cancer among those with the *GSTM1*-null genotype (12). Another isoform of GST (GSTT1) is also involved in carcinogen detoxification and its deletion polymorphism has been suggested to be associated with lung cancer in several studies. In a recent GSEC pooled analysis, the association was not significant for either Asians or Caucasians and no interaction was observed between *GSTT1*-null genotype and smoking on lung cancer (13).

Pooled analyses based on the GSEC data suggest that the effects of these variants tend to differ according to ethnicity possibly because of differences in linkage disequilibrium and environmental exposures. Consequently, gene-environment or gene-gene interactions might differ by ethnic group. Thus, we focused on Asian populations and evaluated the potential role of four selected polymorphisms in the three aforementioned genes (*CYP1A1* $Ile^{462}Val$ and $T^{6235}C$, and null genotypes for *GSTM1* and *GSTT1*) in the development of lung cancer and its specific cell types.

Materials and Methods

Study Population. Subjects were recruited from the International Collaborative Study on GSEC. The design of this collaborative project is explained in detail elsewhere (14). We obtained the original data of 15 case-control studies on genetic polymorphisms in *CYP1A1*, *GSTM1*, or *GSTT1* and risk of lung cancer conducted in Asian populations (15-30). Two studies were excluded due to a sample size of <10 subjects (29) or Caucasian ethnicity (Turkish; Table 1; ref. 30). The participation in GSEC was voluntary, and therefore, some relevant studies were not included in our analysis. The number of subjects included in this pooled analysis was 1,971 cases and 2,130 controls.

Statistical Analysis. All statistical procedures were conducted using Statistical Analysis System version 9.1.3 (SAS Institute) unless otherwise indicated. We estimated the study-specific odds ratios (OR) of lung cancer for each polymorphism using unconditional logistic regression. Results might vary slightly from those reported for some of the published studies because of differences in the inclusion criteria of cases and controls and in the statistical analyses. Heterogeneity among the studies was evaluated by means of the Cochrane Q test and publication bias was assessed by Begg's and Egger's test using STATA version 9. In the pooled analysis, lung cancer risk was estimated with the ORs and 95% confidence intervals (95% CI) by unconditional logistic regression, adjusting for age, sex, and pack-year.

In addition to conducting analyses of all lung cancer, we calculated cell type–specific ORs for the three most prevalent histologic subtypes of lung cancer: adenocarcinoma (n = 905), squamous cell carcinoma (n = 542), and small cell carcinoma (n = 181). Subgroup analyses for other histologic subtypes were not conducted due to small numbers of cases.

Hardy-Weinberg equilibrium for each single nucleotide polymorphism of *CYP1A1* was tested among controls with a Pearson χ^2 and linkage disequilibrium was assessed with D' and r^2. Individual haplotypes for two *CYP1A1* polymorphisms ($Ile^{462}Val$ and $T^{6235}C$) were estimated by expectation-maximization method and the overall difference in haplotype frequency profiles between cases and controls was assessed using the likelihood ratio test. The subjects missing both polymorphisms were excluded in haplotype analysis. The program uses a weighting scheme based on expectation-maximization–derived haplotype frequency estimates. Thus, every haplotype is weighted by the probability of carrying each pair of haplotypes rather than assigning a most likely haplotype to an individual. Missing genotypes result in more low-probability haplotype pairs and

Table 1. Selected characteristics of case-control studies pooled

Author	Ethnicity	Cases (n)	Controls (n)	Reference no.
Kihara et al. (1995)	Japanese	179	259	(15)
Ge et al. (1996)	Chinese	98 (39)*	27 (12)	(16)
Sugimura et al. (1998)	Japanese	260	209	(17)
Persson et al. (1999)	Chinese	80 (35)	123 (45)	(18)
Le Marchand et al. (1998)	Japanese	112 (42)	174 (50)	(19)
Kiyohara et al. (1998, 2000)	Japanese	132 (49)	84	(20, 21)
Lan et al. (2000)	Chinese	122 (43)	122 (43)	(22)
Yin et al. (2001)	Chinese	63 (9)	62 (9)	(23)
Zhao et al. (2001)	Chinese	233 (233)	190 (190)	(24)
Sunaga et al. (2002)	Japanese	198	152	(25)
Wang et al. (2003)	Chinese	112 (40)	119 (40)	(26)
Lee et al. (2006)	Korean	171	196	(27)
Pisani et al. (2006)	Thai	211 (71)	413 (158)	(28)
Total		1,971 (635)	2,130 (591)	

NOTE: One study with <10 subjects [Dresler et al. (29)] and Caucasian subjects [Pinarbasi et al. (30)] was excluded.
*Number of female subjects.

Table 2. Characteristics of subjects (1,971 cases and 2,130 controls)

	Cases, *n* (%)	Controls, *n* (%)	*P*	OR (95% CI)*
Age (y)				
<50	219 (11.1)	444 (20.9)	0.0001	
50-59	501 (25.4)	638 (30.0)		
60-69	718 (36.5)	599 (28.2)		
70-79	447 (22.7)	376 (17.7)		
≥80	85 (4.3)	70 (3.3)		
Mean (±SD)	62.6 (±10.7)	58.4 (±13.2)	0.0001	
Sex				
Male	1,336 (67.8)	1,537 (72.2)	0.002	
Female	635 (32.2)	591 (27.8)		
Smoking status				
Never	462 (24.9)	764 (38.3)	0.0001	Reference
Ever	1,396 (75.1)	1,230 (61.7)		2.29 (1.94-2.70)
Missing	113	136		
Pack-years in ever smokers				
0 < pack-year <35	468 (42.4)	640 (64.6)	0.0001	1.54 (1.28-1.36)
Pack-year ≥35	636 (57.6)	351 (35.4)		4.36 (3.51-5.35)
Missing	292	239		
Mean (±SD)	66.8 (±146.5)	49.4 (±107.9)	0.002	
Pathologic type				
AD	905 (50.2)			
SQ	542 (30.1)			
SM	181 (10.0)			
Other cell types	174 (9.7)			
Missing	169			

Abbreviations: AD, adenocarcinoma; SQ, squamous cell carcinoma; SM, small cell carcinoma.
*ORs were adjusted for age and sex.

each haplotype is weighted as such. An unconditional logistic regression model was used to estimate the effect of individual haplotypes by fitting an additive model, adjusting for sex, age, and pack-year.

Gene-smoking interactions (i.e., the modification of increasing pattern of lung cancer risk as the pack-year increases by different genotype) were evaluated by the significance of the coefficient of product term

Table 3. *CYP1A1* genotypes and lung cancer risk by histologic types

	Controls, *n* (%)	All cases, *n* (%)	OR (95% CI)*	AD, *n* (%)	OR (95% CI)*	SQ, *n* (%)	OR (95% CI)*	SM, *n* (%)	OR (95% CI)*
Ile^{462}Val	*n* = 1,096	*n* = 910		*n* = 337		*n* = 343		*n* = 121	
Ile/Ile	609 (55.6)	502 (55.2)	Reference	188 (55.8)	Reference	180 (52.5)	Reference	72 (59.5)	Reference
Ile/Val	421 (38.4)	329 (36.2)	0.88 (0.71-1.08)	117 (34.7)	0.94 (0.69-1.27)	132 (38.5)	1.06 (0.78-1.45)	41 (33.9)	0.80 (0.50-1.28)
Val/Val	66 (6.0)	79 (8.7)	1.06 (0.71-1.56)	32 (9.5)	1.53 (0.92-2.56)	31 (9.0)	1.01 (0.55-1.85)	8 (6.6)	0.60 (0.22-1.67)
P_{trend}			0.57		0.37		0.78		0.21
Ile/Ile or Ile/Val	1,030 (94.0)	831 (91.3)	Reference	305 (90.5)	Reference	312 (91.0)	Reference	113 (92.4)	Reference
Val/Val	66 (6.0)	79 (8.7)	1.14 (0.76-1.72)	32 (9.5)	1.57 (0.96-2.59)	31 (9.0)	1.14 (0.76-1.72)	8 (6.6)	0.65 (0.24-1.79)
T^{6235}C (MspI)	*n* = 953	*n* = 729		*n* = 284		*n* = 261		*n* = 95	
TT	333 (34.9)	241 (33.1)	Reference	106 (37.3)	Reference	75 (28.7)	Reference	36 (37.9)	Reference
TC	449 (47.1)	341 (46.8)	1.08 (0.84-1.39)	125 (44.0)	1.08 (0.84-1.39)	120 (46.0)	1.42 (0.96-2.09)	45 (47.4)	1.10 (0.65-1.86)
CC	171 (17.9)	147 (20.2)	1.13 (0.82-1.56)	53 (18.7)	1.13 (0.82-1.56)	66 (25.3)	1.97 (1.26-3.07)	14 (14.7)	0.73 (0.36-1.51)
P_{trend}			0.43		0.43		0.003		0.52
TC or CC	620 (65.1)	488 (67.0)	1.10 (0.86-1.39)	178 (62.7)	1.10 (0.86-1.39)	186 (71.3)	1.58 (1.10-2.27)	50 (52.6)	0.98 (0.60-1.62)
Haplotype†	*n* = 1,172 %	*n* = 979 %		*n* = 361 %		*n* = 385 %		*n* = 123 %	
Ile-T	56	52	Reference	55	Reference	49	Reference	57	Reference
Ile-C	19	21	1.39 (1.12-1.71)	18	0.99 (0.73-1.34)	24	2.10 (1.58-2.80)	19	1.29 (0.83-2.01)
Val-T	2	4	3.41 (1.78-6.53)	4	4.84 (2.32-10.1)	4	3.75 (1.70-8.27)	1	0.37 (0.02-8.06)
Val-C	23	23	0.96 (0.79-1.15)	23	0.94 (0.73-1.12)	24	1.06 (0.81-1.38)	23	0.89 (0.60-1.31)
$P_{omnibus}$‡			0.0001		0.0003		0.0001		0.40

*ORs were adjusted for age (<50, 50-59, 60-69, 70-79, and ≥80 y), sex, and pack-year.
†Subjects missing for both *CYP1A1 Ile^{462}Val* and *T^{6235}C (MspI)* data were excluded.
‡*P* value from the test of overall difference of haplotype distribution between cases and controls.

Table 4. *GSTM1* and *GSTT1* genotypes and lung cancer risk by histologic types

	Controls, *n* (%)	All cases, *n* (%)	OR (95% CI)*	AD, *n* (%)	OR (95% CI)*	SQ, *n* (%)	OR (95% CI)*	SM, *n* (%)	OR (95% CI)*
GSTM1	*n* = 1,604	*n* = 1,419		*n* = 760		*n* = 333		*n* = 169	
Present	713 (44.5)	589 (41.5)	Reference	332 (43.7)	Reference	124 (37.2)	Reference	59 (41.3)	Reference
Null	891 (55.6)	830 (58.5)	1.11 (0.95-1.29)	428 (56.3)	0.99 (0.82-1.19)	209 (62.8)	1.36 (1.05-1.77)	84 (58.7)	1.27 (0.88-1.83)
GSTT1	*n* = 1,024	*n* = 1,135		*n* = 579		*n* = 248		*n* = 71	
Present	538 (52.5)	579 (51.0)	Reference	300 (51.8)	Reference	141 (56.9)	Reference	25 (35.2)	Reference
Null	486 (47.5)	556 (49.0)	1.02 (0.84-1.24)	279 (48.2)	1.00 (0.80-1.26)	107 (43.2)	0.87 (0.62-1.21)	46 (64.8)	1.36 (0.99-1.86)

*ORs were adjusted for age (<50, 50-59, 60-69, 70-79, and ≥80 y), sex, and pack-year.

genotype*pack-year in the model. The test was equal to evaluate the difference of the slopes of two fitted lines stratified by categorized genotypes. Additionally, we tested the significance of the product term in the model without main effect term of genotype, which assumes that if there is no exposure to cigarette smoking, there is no difference in the risk of lung cancer between genotypes (27, 31). The assumption of no genotype effect when there is no smoking exposure was equal to common intercept assumption for two fitted lines by genotypes.

Results

The distributions by age, sex, smoking status, and cell types of the 1,971 lung cancer cases and 2,130 controls are presented in Table 2. The mean age was 62.6 (±10.7 years) in cases and 58.4 (±13.2 years) in controls ($P = 0.0001$). The proportion of ever smokers was much greater in cases (75.1%) than in controls (61.7%; $P = 0.0001$). In terms of cell types, adenocarcinoma (50.2%) and squamous cell carcinoma (30.1%) were the most common.

Genotype frequencies of *CYP1A1* $Ile^{462}Val$ and $T^{6235}C$ were consistent with Hardy-Weinberg equilibrium in the control group ($P > 0.35$) and the two polymorphisms were in moderate linkage disequilibrium ($D' = 0.86$ and $r^2 = 0.35$). The variant allele frequencies of the three polymorphisms (*CYP1A1* ^{462}Val, 0.25; ^{6235}C, 0.42; and *GSTT1* null, 0.48) in the controls were higher compared with those of Caucasian or African populations (13, 32). The frequency of the *GSTM1* null (0.56) was similar to that of Caucasians but higher compared with Africans (32). The *CYP1A1* ^{6235}C variant was associated with squamous cell lung cancer (*TC* versus *TT*: OR, 1.42; 95% CI, 0.96-2.09; *CC* versus *TT*: OR, 1.97; 95% CI, 1.26-3.07; $P_{trend} = 0.003$; Table 3). The *CYP1A1* ^{462}Val variant was moderately associated with adenocarcinoma (*Val/Val* versus *Ile/Ile* or *Ile/Val*: OR, 1.57; 95% CI, 0.96-2.59).

In haplotype analysis, ^{462}Val-^{6235}T and *Ile*-*C* haplotypes were associated with lung cancer risk with reference to the *Ile*-*T* haplotype (OR, 3.41; 95% CI, 1.78-6.53 and OR, 1.39; 95% CI, 1.12-1.71, respectively). An omnibus test showed that the distribution of the *CYP1A1* haplotypes was significantly different between all lung cancer cases and controls ($P = 0.0001$). In subgroup analysis, the difference was also significant for adenocarcinoma ($P = 0.0003$) and squamous cell carcinoma ($P = 0.0001$) and not for small cell carcinoma ($P = 0.40$).

The *GSTM1*-null genotype significantly increased squamous cell lung cancer risk (OR, 1.36; 95% CI, 1.05-1.77), and the *GSTT1*-null genotype was moderately associated only with small cell lung cancer risk (OR, 1.36; 95% CI, 0.99-1.86; Table 4). Analysis of combined genotypes did not reveal associations beyond what was apparent in the single polymorphism analyses (data not shown).

When the interaction was evaluated with smoking, increasing trend of lung cancer risk as pack-year increased was much stronger among those with the *CYP1A1* 6235 *TC/CC* genotype compared with those with *TT* genotype ($P_{interaction} = 0.001$; Fig. 1). Although the association between smoking and lung cancer was stronger among those with the *GSTM1*-null genotype compared with the present type, it was only marginally significant with the assumption of no genotype effect in the absence of the smoking exposure ($P_{interaction} = 0.08$). Significant interactive effect with smoking has not been observed for *GSTT1*.

There was no evidence of significant heterogeneity among studies or of publication bias for all four polymorphisms investigated in our study; we found only moderate heterogeneity for the effect of *CYP1A1* $^{462}Val/Val$ compared with *Ile/Ile* ($P = 0.08$), and all Begg's and Egger's tests were not significant ($P \geq 0.2$ and 0.3, respectively).

Discussion

Our results suggest that the *CYP1A1* polymorphisms ($Ile^{462}Val$ and $T^{6235}C$) and the *GSTM1*-null genotype are associated with lung cancer risk, especially for squamous cell carcinoma, in Asian populations. In addition, the association of smoking with lung cancer was significantly modified by the *CYP1A1* $T^{6235}C$ polymorphism in our study.

A significant interactive effect between the *CYP1A1* ^{6235}C allele and smoking is consistent with the results of previous pooled analysis that the stronger association between the ^{6235}C allele and lung cancer was found among ever smokers (5). The previous pooled analysis for the *GSTM1*-null genotype conducted by Benhamou et al. (12) found a nonsignificant elevated lung cancer risk among Asians, especially among heavy smoker (>40 pack-years). Likewise, our extended analysis with additional Asian populations also observed a moderate elevation of overall lung cancer risk by the *GSTM1* deletion and moderate interaction with smoking. On the

other hand, stronger effect of *CYP1A1* ^{462}*Val* found in previous pooled analysis among nonsmokers (6) was not observed in Asian populations investigated in our study.

Le Marchand et al. (19) hypothesized that genetic susceptibility to polycyclic aromatic hydrocarbons (based on high-risk genotypes for *CYP1A1* and *GSTM1*) predominantly causes squamous cell carcinoma. In the multiethnic study conducted by Le Marchand et al. (19), *CYP1A1* ^{6235}C allele was associated with a 3.1-fold risk of squamous cell carcinoma when combined with a *GSTM1* deletion. Decreasing trend of squamous cell carcinoma, relative to the increase in adenocarcinoma, associated with filter-tipped cigarettes in developed country indirectly supports this hypothesis (33). The increased risk of squamous cell carcinoma in relation with the *GSTM1*-null genotype observed in our study is consistent with the results of previous studies, including those of a meta-analysis (10, 19, 34, 35). The effect of the *CYP1A1* ^{6235}C allele, especially when combined with a *GSTM1*-null genotype, also tended to be associated with a higher risk of squamous cell carcinoma among Asians (5); in our study, *CYP1A1* TC or CC genotype was associated with significant elevation of squamous cell carcinoma risk compared with TT genotype (OR, 1.6) and Ile-C haplotype was significantly associated with squamous cell carcinoma risk (OR, 2.1).

BPDE is known to induce G:C to T:A transversion mutations in the hotspot codons of the *p53* tumor suppressor gene (36), which is found more frequent in squamous cell carcinoma than in adenocarcinoma (37). Cigarette smoke is also known to be causally related to BPDE-DNA adducts (38, 39), and BPDE-DNA adduct

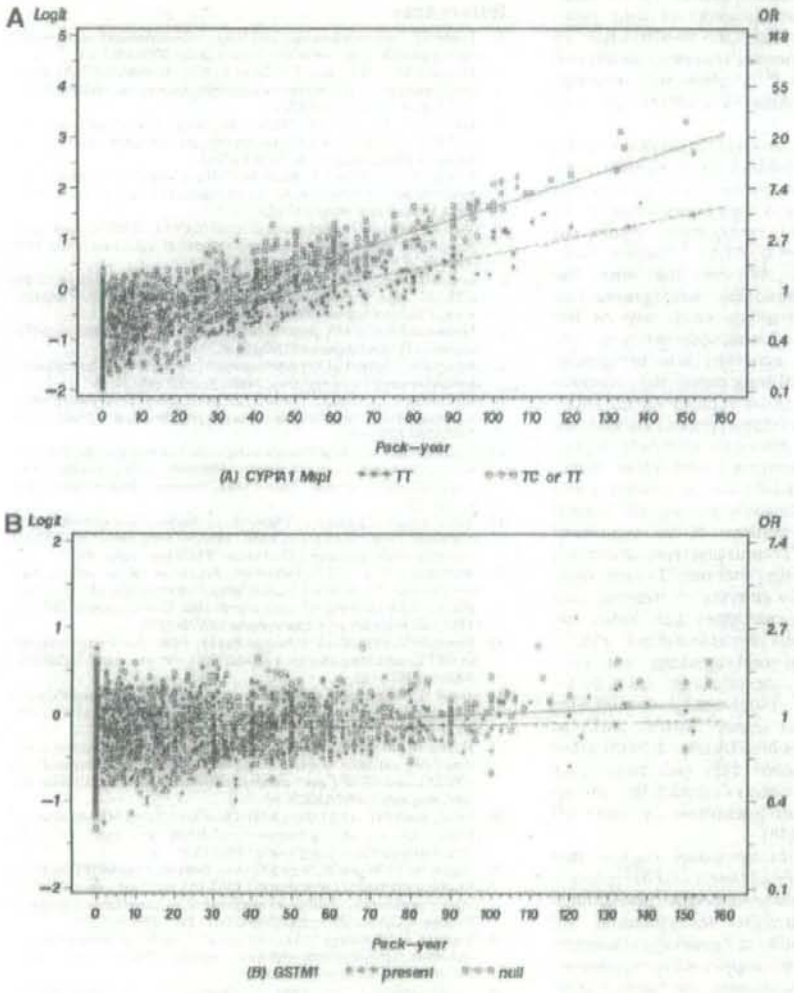

Figure 1. The smoking effect on lung cancer stratified by the *CYP1A1* $T^{6235}C$ *(MspI)* (**A**) and *GSTM1* null/present (**B**). When the interaction was evaluated with smoking, increasing trend of lung cancer risk as pack-year increased was stronger among those with the *CYP1A1* 6235 TC/CC genotype compared with those with TT genotype ($P_{interaction}$ = 0.001). Although the association between smoking and lung cancer was stronger among those with the *GSTM1*-null genotype compared with the present type, it was only marginally significant with the assumption of common intercept ($P_{interaction}$ = 0.08).

level is elevated in the lung parenchyma of smokers with *GSTM1*-null genotype (40). Moreover, the combined genotypes of *CYP1A1* ^{462}*Val* and *GSTM1* null have been associated with increased adduct level in lung tissues of squamous cell carcinoma patients (41). Thus, it is speculated that our finding of an association between *GSTM1* and *CYP1A1* polymorphisms with the risk of squamous cell carcinoma is related to polycyclic aromatic hydrocarbon exposure derived from smoking because polycyclic aromatic hydrocarbons are primarily metabolized by *CYP1A1* and *GSTM1*. The greater effects observed among smokers also support this smoking-related etiology of squamous cell carcinoma in Asian population.

Our study is the largest pooled analysis conducted for Asian populations to evaluate the role of polymorphisms in carcinogen-metabolizing genes (i.e., *CYP1A1*, *GSTM1*, and *GSTT1*) in lung cancer development. We simultaneously evaluated the potential effect of four polymorphisms on lung cancer and the modification of those effects by smoking exposure reporting significant interaction between *CYP1A1* ^{6235}C allele and smoking. Subtype-specific results in Asian population are also noteworthy.

However, our study has several limitations to be considered. First, not all published Asian studies were included in this study. However, there was no evidence of significant publication bias for this pooled analysis. In terms of heterogeneity, only marginally significant heterogeneity was found for *CYP1A1* ^{462}*Val/Val* compared with *Ile/Ile* (P = 0.08). We note that when the adjusted values were considered, the heterogeneity did not remain. Other limitation of our study may be the relatively small sample size in subgroup analyses. We found that the *GSTT1*-null genotype was marginally associated only with small cell lung cancer risk, whereas no association with lung cancer was observed for either Asians or Caucasians in the previous pooled analysis for *GSTT1*-null genotype (13). Although relatively higher variant allele frequencies, compared with other ethnic groups (13, 32), may compensate for the relative small sample size in terms of statistical power, we cannot exclude chance for the explanation of the significant association between the *GSTT1*-null genotype and small cell lung cancer risk, considering that only 71 cases were available. Sizable exclusion of subjects for missing data on smoking and pathologic subtypes also limits the conclusion from our results for interactive effects between the polymorphisms and smoking, and subtype-specific analysis. Thus, our findings need to be replicated in a larger study. Future study should also include the measurement of dietary factors, such as isothiocyanates, which are involved in the detoxification of tobacco-related carcinogens (42) and may have protective effects on lung cancer especially among smokers or those with *GST*-null genotypes, as observed in a Chinese population (24, 43).

In summary, the results of our study suggest that genetic polymorphism in *CYP1A1* and *GSTM1* plays a role in lung cancer susceptibility in Asian populations and that the effects are strongest for squamous cell carcinoma. Although our results are generally consistent with previous studies and are supported by epidemiologic and experimental observations, additional large studies are needed to help to elucidate the role of genetic polymorphisms in xenobiotic-metabolizing genes in lung cancer development. The interaction between environmental exposure other than smoking (e.g., indoor coal combustion) and these polymorphisms still remains to be evaluated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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EphB1 Is Underexpressed in Poorly Differentiated Colorectal Cancers

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Key Words
EphB1 · Colorectal cancer · Quantitative real-time RT-PCR · Immunohistochemical staining

Abstract
Background: Over- or underexpression of certain Eph receptors has been associated with tumorigenesis of some types of cancer. *EphB1* is a member of receptor tyrosine kinases of the EphB subfamily involved in the development, progress and prognosis of colorectal cancers. The expression levels of EphB1 in colon cancer cell lines and human colorectal carcinoma specimens were determined and association of EphB1 expression with clinicopathological parameters was analyzed. ***Methods:*** Quantitative real-time reverse transcription polymerase chain reaction and immunohistochemistry were used. ***Results:*** The EphB1 transcript is expressed in all colon cancer cell lines tested. However, there is marked variability in the expression of the EphB1 transcripts and proteins among colorectal carcinoma specimens. Reduced expression of EphB1 in colorectal cancers more often occurred in poorly differentiated and mucinous adenocarcinomas than in well- and moderately differentiated adenocarcinomas. Further, cancer cells with a low level of EphB1 protein showed more invasive power. ***Conclusion:*** Our data indicate that *EphB1* may have roles in the pathogenesis and development of colorectal cancer.

Introduction

The Eph family, named for its expression in an erythropoietin-producing human hepatocellular carcinoma cell line [1], is the largest subfamily of the receptor tyrosine kinases and includes at least 14 distinct receptors and 8 distinct ligands. Both the Ephs (receptors) and ephrins (ligands) are divided into 2 groups, the A and B subfamilies [2]. The interactions between Eph receptors and ephrin ligands play important roles in vascular development, tissue border formation, cell migration, axon guidance and angiogenesis [3–7]. Unlike other families of the receptor tyrosine kinases, which bind to soluble ligands, Eph receptors interact with cell membrane-bound ephrin ligands. Moreover, these receptor-ligand interactions activate a bidirectional signaling pathway through both the Eph receptors and ephrin ligands. Some receptors of the Eph gene have been found overexpressed in human tumors, including neuroblastoma, lung, gastric, esophageal, breast and colorectal cancer. Overexpression of Eph receptors could be correlated to altered tumor behaviors, such as increased metastatic potential and poor patient outcome. But more recently, Eph receptors and ephrins have been recognized as being differentially expressed in various human tumors. Our previous studies showed

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that EphA7 was underexpressed in colorectal cancers, and we proved that aberrant methylation of the 5′CpG islands is the main mechanism that leads to the down-regulation of *EphA7* [8]. We also found that EphA7 receptor is differentially expressed in gastric carcinoma [9].

Colorectal cancer is the second most common type of cancer in the Western world and its incidence has recently also markedly increased in other countries such as China. EphB/ephrinB signaling is essential for the correct formation of crypts and villi in the intestinal epithelium [10–12]. Increasing data have shown that the EphB subfamily is involved in the carcinogenesis of colorectal cancer. Among the Eph family genes, relatively little attention has been directed toward *EphB1* in human colorectal cancer, and the potential role of *EphB1* in human colorectal cancer has not been addressed. *EphB1* was first identified in a rat brain cDNA expression library. In human, EphB1 was preferentially highly expressed in normal brain, testis and colon. Expression of EphB1 in certain human tumors has been investigated. However, the results were inconsistent. In order to investigate the role of *EphB1* in tumorigenesis of colorectal cancer, quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry were used to determine expression of EphB1 in human colon cancer cell lines, colorectal adenomas and colorectal cancer tissues. Our data show that underexpression of EphB1 often occurs in colorectal cancer patients with poorly differentiated tumor. And cancer cells with low level of EphB1 protein show more invasive power. The results indicate that *EphB1* plays a role in the development and prognosis of colorectal cancer.

Materials and Methods

Colon Cancer Cell Lines and Tissue Specimens

The colon cancer cell lines SW480, DLD1, HT29, HCT116 and SW620 were used in the present study. The cells were routinely maintained in Dulbecco's modified Eagle medium (NISSUI Pharmaceutical Co.) supplemented with 1 mmol/l L-glutamine, 10% fetal bovine serum (Life Technologies Inc.), 100 U/ml of penicillin G and 100 mg/ml of streptomycin. The cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

A total of 15 colorectal adenomas, including 6 of low and 9 of high grade, 78 colorectal carcinoma and uninvolved normal mucosa specimens were obtained from surgical resections performed at the Nanjing Jinling Hospital between 2005 and 2006, as part of a study approved by the Research Ethics Board of the Nanjing Jinling Hospital. The distribution of the tumors by sites of origin was as follows: cecum and ascending colon, 14 tumors; sigmoid colon, 9 tumors; rectum, 51 tumors; others, 4 tumors. Formalin-fixed and paraffin-embedded tumor tissue sections were stained with hematoxylin and eosin and examined histologically. The clinicopathological characteristics of the 78 colorectal patients are shown in table 1. The tissue samples were immediately frozen in liquid nitrogen and stored at –80°C for the extraction of total RNA and DNA. All tissue specimens were evaluated pathologically. No patients had received irradiation or cancer chemotherapy prior to resection.

Table 1. Characteristics of 78 patients with colorectal carcinoma

Variable	n
Male:female	47:31
Age	
≤55 years	38
>55 years	40
Location	
Rectum and sigmoid colon	60
Others	18
Depth of wall invasion	
Mucosae and submucosa	4
Muscularis propria	22
Subserosa and serosa	52
Tumor differentiation	
Well-differentiated adenocarcinoma	12
Moderately differentiated adenocarcinoma	47
Poorly differentiated adenocarcinoma	7
Mucinous adenocarcinoma	12
Stage (TNM)	
I	23
II	26
III+IV	29
Lymph node metastasis	
Absent	49
Present	29

Quantitative Real-Time RT-PCR

To detect the expression of the EphB1 transcript in carcinoma samples, a quantitative real-time RT-PCR was subjected to the cDNAs. The reactions were performed in triplicate. The sense and antisense primers and TaqMan probe for *EphB1* were designed according to the mRNA sequence (GenBank accession No. NM_004441). Amplification of PCR fragments spanning different exons was used to prevent contamination of genomic DNA. The sense primer was 5′-GCGATGGCCCTGGATTATCTAC-3′ and antisense primer was 5′-GCAGTAGCCGTTCTGGTGTC-3′. The PCR products were 92 bp long. The TaqMan probe was 5′-(FAM) TCCTCCTGGCATCCGCAGTGGCT (Eclipse)-3′. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)* was used as an internal control (GenBank accession No. NM_002046). The sense primer was 5′-CCAGGTGGTCTCCTCTGACTT-3′ and the antisense primer was 5′-GTTGCTGTAGCCAAATTCGTTGT-3′. The PCR products were 130 bp long. The probe was 5′-(FAM) AACAGCGACACCCACTCCTCCACC (Eclipse)-3′. The values of EphB1 mRNA expression were normalized using the GAPDH expression. Primer sets and probes were synthesized by TaKaRa Biotechnology Inc. The reaction mixture

included 1× buffer, 200 μmol/l of deoxy-ribonucleoside triphosphates (dNTPs; Invitrogen), 0.3 μmol/l of sense and antisense primers, 1 U of Takara ExTaq Hotstart Taq (TaKaRa Biotechnology), 0.6 μmol/l of 5-carboxy-X-rhodamine reference dye (Invitrogen) and 2 μl of cDNA. The PCR cycle involved 2 min at 95°C followed by 40 amplification cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and elongation at 72°C for 1 min.

Immunohistochemical Staining

Sections from the surgical specimens that were fixed in 10% formalin and embedded in paraffin were studied. Immunohistochemical staining was performed according to the standard method. Briefly, each 2-μm tissue section was deparaffinized and rehydrated. After rehydration using a gradient of ethanol concentrations, the sections were autoclaved in 10 mmol/l citrate buffer (pH 6.0) at 120°C for 2 min for antigen retrieval, then naturally cooled to 30°C and washed with phosphate-buffered saline (PBS, pH 7.3). The sections were incubated with an anti-EphB1 polyclonal antibody (Abgent) at a dilution of 1:100 in antibody diluent solution (Zymed, Invitrogen) at 4°C overnight, followed by washing with PBS. The sections were then incubated with secondary antibody (Dako) for 30 min at room temperature. Color development was performed with 3,3′-diaminobenzidine. Nuclei were lightly counterstained with hematoxylin.

Evaluation of Staining for EphB1

Two pathologists independently assessed the immunostained slides. Any difference in the immunohistochemical scores was resolved by consensus. Immunohistochemical staining of both normal mucosa and cancer cells was assessed according to both the intensity and the proportion of cells that were stained. Staining intensity was recorded as 0 for no staining, 1 for weak staining, 2 for moderate staining and 3 for strong staining. The percentage of positive cells was classified semiquantitatively as 0 for tissue specimens without staining, 1 for fewer than 25% of cells stained, 2 for 25–50% of cells stained and 3 for more than 50% of the tissue stained. Scores for expression and percentage of positive cells were added. The EphB1 expression was assessed by comparing the scores for tumor cells and adjacent normal mucosa cells.

Methylation-Specific PCR

Genomic DNA was modified by sodium bisulfite, as described by Clark et al. [13, 14]. Primers were designed using MethPrimer software on the Internet (http://www.urogene.org/methprimer/) to discriminate between methylated and unmethylated alleles following sodium bisulfite treatment. Two-microliter aliquots were amplified in a 30-μl reaction mixture consisting of 1× buffer (10 mM Tris-HCl, 2.0 mM $MgCl_2$, 50 mM KCl, pH 8.3), 1 U Takara ExTaq Hotstart Taq, 260 μM dNTPs and 0.3 μM of the primer sets. The PCR conditions were as follows: 95°C for 2 min, then 35 cycles of 94°C for 30 s, 55°C (for detection of methylated DNA) or 56°C (for detection of unmethylated DNA) for 30 s, 72°C for 1 min and finally 10 min at 72°C. Methylation-specific primer set was: 5′-TGCGGTTTTTCGAGAGTATTAC-3′ (forward) and 5′-GAAACCGACCAAACTAACGC-3′ (reverse). Unmethylation-specific primer set was: 5′-TGGTTTTTTGAGAGTATTATGA-3′ (forward) and 5′-AACAAAACCAACCAAACTAACA-3′ (reverse). The PCR products were 248 bp long. The PCR products were run on 8% nondenaturing polyacrylamide gel, followed by ethidium bromide staining.

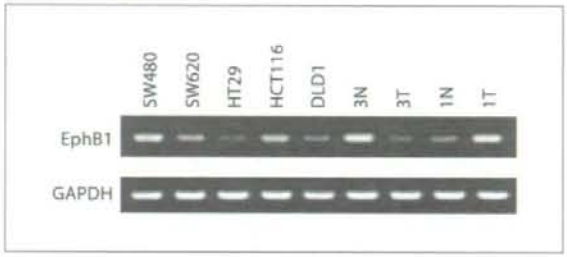


Fig. 1. Expression of EphB1 in colon cancer cell lines and representative examples of colorectal cancer.

Statistical Analysis

The statistical significance of intergroup differences was analyzed using the χ^2 test. All statistical analyses were performed using SPSS 11.5 software (SPSS Inc.). For all statistical tests, two-sided $p < 0.05$ was considered statistically significant.

Results

Expression of the EphB1 Transcript in Colon Cancer Cell Lines

Expression of EphB1 in the colon cancer cell lines SW480, SW620, DLD1, HT29 and HCT116 was assessed using quantitative real-time RT-PCR. The EphB1 transcript was differentially expressed in colon cancer cell lines, EphB1 expression is high in SW480 and low in HT29 (fig. 1).

Expression of the EphB1 Transcript in Colorectal Carcinoma Specimens

Expression of the EphB1 transcription was detected using quantitative real-time RT-PCR in 78 colorectal carcinoma specimens that contained paired uninvolved normal mucosa and tumor tissue. Colorectal carcinoma samples showed marked interspecimen variability in their levels of EphB1 expression. The expression level of EphB1 in colorectal carcinoma tissues was compared with that in paired uninvolved normal mucosa tissues and classified as A, B or C according to the ratio of the 2: A = uninvolved normal mucosa-to-tumor ratio greater than 2 (N/T >2); B = uninvolved normal mucosa-to-tumor ratio less than 0.5 (N/T <0.5); C = normal mucosa-to-tumor ratio between 0.5 and 2 (N/T 0.5–2; table 2). Downregulation of EphB1 (class A) was observed in 27 (34.6%) colorectal carcinoma specimens, while overexpression of EphB1 (class B) was observed in 36 (46.2%) samples.

Table 2. Correlation between EphB1 transcript expression and clinicopathologic parameters

	N/T ≥2	N/T 2–0.5	N/T ≤0.5	p
Overall	27	15	36	
Sex				
Male	18	10	19	
Female	9	5	17	0.458
Age				
≤55 years	13	9	16	
>55 years	14	6	20	0.597
Location				
Rectum and sigmoid colon	23	11	26	
Others	4	4	10	0.45
Depth of wall invasion				
Mucosae and submucosa[1]	2	1	1	
Muscularis propria	8	3	11	
Subserosa and serosa	17	11	24	0.751
Pathological classification				
Well and moderate	18	9	32	
Poor and mucinous	9	6	4	0.037
Clinical stage (TNM)				
I	9	4	10	
II	7	6	13	
III+IV	11	5	13	0.894
Lymphatic metastases				
Negative	17	10	22	
Positive	10	5	14	0.932
Dukes				
A+B	16	10	22	
C	11	5	14	0.892

[1] The number was not used because it was too small.

Table 3. Correlation between EphB1 protein expression and clinicopathologic parameters

	Down-regulation	No difference	Up-regulation	p
Overall	39	15	15	
Sex				
Male	29	10	5	
Female	10	5	10	0.019
Age				
≤55 years	20	9	4	
>55 years	19	6	11	0.152
Location				
Rectum and sigmoid colon	27	13	14	
Others	12	2	1	0.106
Depth of wall invasion				
Mucosae and submucosa[1]	2	2	0	
Muscularis propria	8	3	9	
Subserosa and serosa	29	10	6	0.02
Tumor differentiation				
Well and moderate	25	13	14	
Poor and mucinous	14	2	1	0.043
Clinical stage (TNM)				
I+II	27	8	9	
III+IV	12	7	6	0.521
Lymphatic metastases				
Absent	27	8	9	
Present	12	7	6	0.521

[1] The number was not used because it was too small.

Correlation between EphB1 Transcript Expression and Clinicopathological Parameters

Table 2 shows the correlation between clinical variables and the expression of the EphB1 transcript. The transcription level of EphB1 was significantly related to the differentiation of the patients ($p = 0.037$). The EphB1 transcript is more often reduced in poorly differentiated carcinomas. There was no significant association between EphB1 transcript expression and sex, age, tumor location, clinical stage, depth of wall invasion and lymph node metastasis.

Expression of EphB1 Protein in Colorectal Adenoma and Carcinoma Specimens

A total of 15 colorectal adenoma, 69 colorectal carcinoma and normal adjacent uninvolved mucosa specimens were immunohistochemically stained with a specific polyclonal *EphB1* antibody. Five samples of low-grade and 8 samples of high-grade adenomas were positively stained; 1 sample of low-grade and 1 sample of high-grade carcinoma were negative. In all samples of normal colon, EphB1 protein expression was most intense at the base of the crypt with expression declining to the luminal epithelium (fig. 2a). The expression level of EphB1 protein differed between colorectal cancer cells, and heterogeneous staining in the same slide was observed (fig. 2c). However, the colorectal adenoma cells were stained homogenously (fig. 2b). The *EphB1* immunoreactivity was observed mainly in golgiosome, cytoplasm and rarely in membrane. Of 69 colorectal carcinoma tissue samples, the EphB1 protein expression was downregulated in 39 (56.5%) and upregulated in 15 (21.7%) tumor samples.

Association of the EphB1 Protein Expression with Clinicopathological Parameters

Table 3 shows the correlation between EphB1 protein expression and clinicopathological characteristics. The

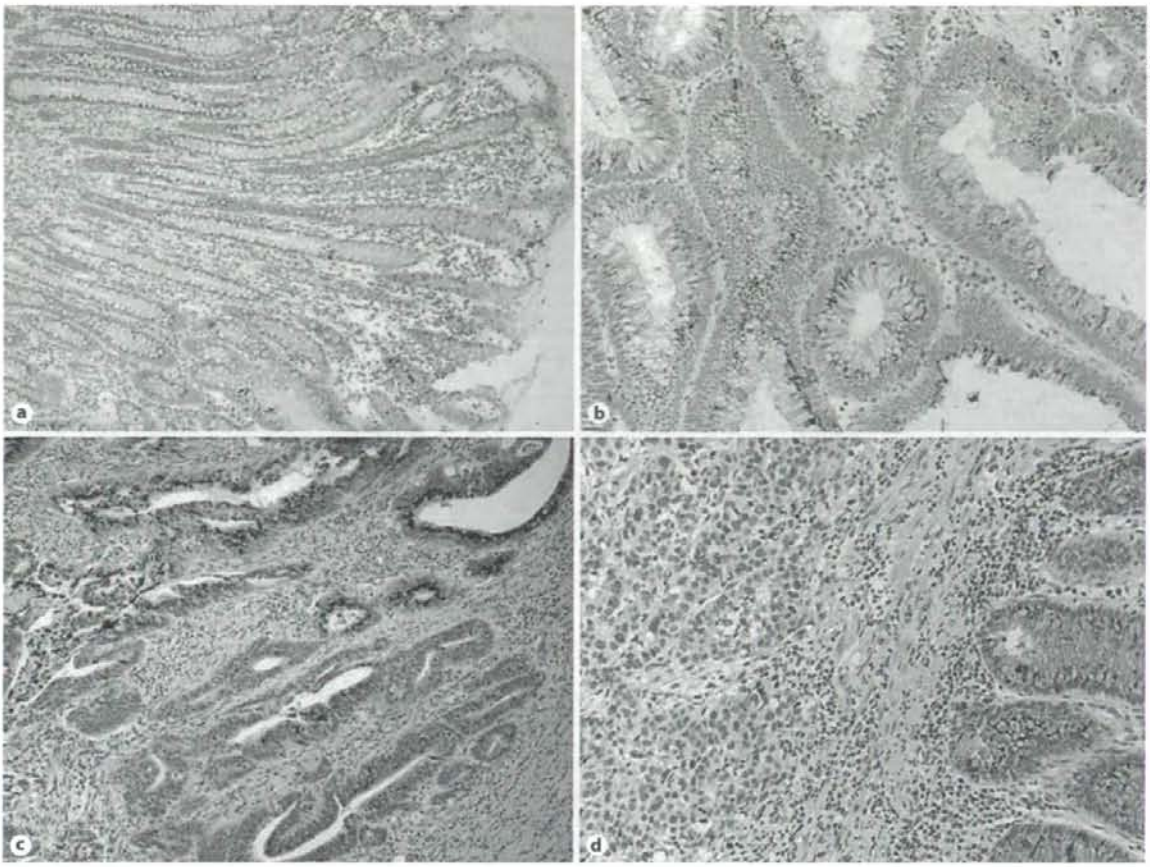


Fig. 2. Expression of EphB1 protein was analyzed by specific polyclonal anti-EphB1 antibody. **a** EphB1 protein expression was most intense at the base of the crypt with expression declining to the luminal epithelium. **b** EphB1 was homogenously stained in colorectal adenomas. **c** EphB1 protein was not uniformly expressed in colorectal cancer cells. **d** Downregulation of EphB1 in colorectal cancer.

EphB1 protein was significantly reduced in male patients ($p = 0.019$) and in those with poor differentiation and mucinous adenocarcinomas or tumors ($p = 0.043$). The colorectal cancer cells with low levels of EphB1 protein more often invaded to serosa and subserosa ($p = 0.020$). Expression of EphB1 protein was not related to other clinicopathological characteristics.

No Methylation of EphB1 in Colorectal Carcinoma Specimens

The methylation status of the *EphB1* promoter-associated 5′CpG island was assessed by methylation-specific PCR. Unmethylated DNA was detected in all 35 sodium bisulfite-treated DNA samples used in this study, however, no methylated DNA of *EphB1* was found.

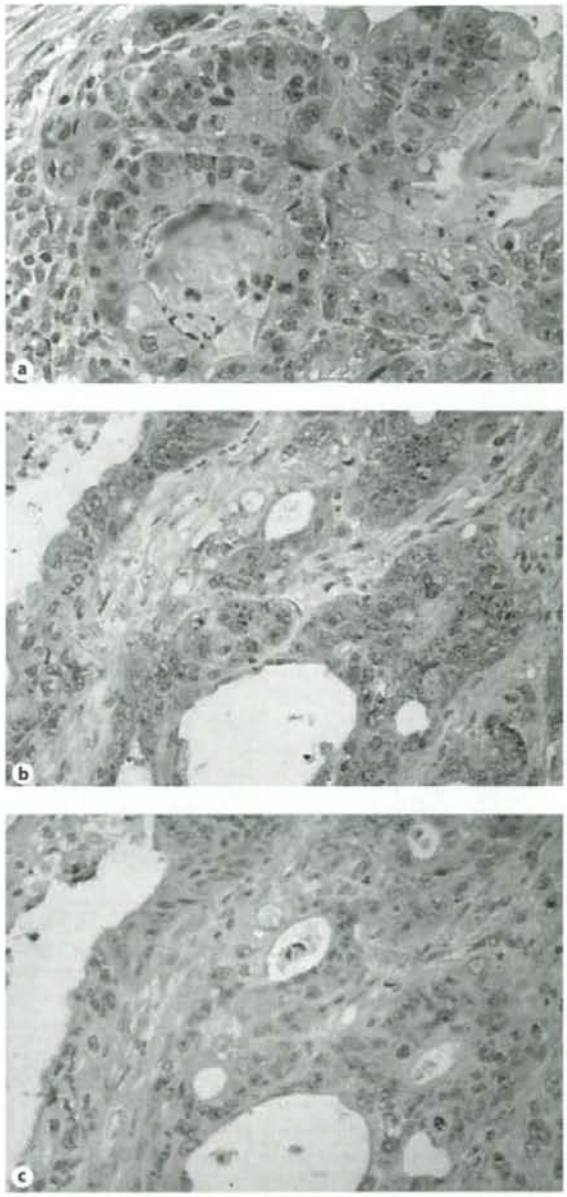
Discussion

The Eph receptors are the largest family of receptor tyrosine kinases, which are involved in cell proliferation, differentiation, migration and other functions. The Eph genes also have important physiologic roles in the intestinal epithelium. In the intestine, epithelia stem

cells reside at the bottom of crypts that are formed by the convolution of the epithelial sheet. Wnt proteins are present at the bottom of crypts and interact with Wnt receptors in epithelial cells. Cytoplasmic β-catenin levels are normally kept low through continuous proteasome-mediated degradation. When epithelial cells receive Wnt signals, the degradation pathway is inhibited, and consequently β-catenin accumulates in the cytoplasm and nucleus. Nuclear β-catenin interacts with transcription factors such as lymphoid enhancer-binding factor 1/T cell-specific transcription factor to affect transcription. As the direct transcriptional target of the β-catenin/T cell-specific transcription factor complex, expression of EphB2 and EphB3 genes is inversely controlled along the crypt-villus axis [12]. EphB receptors have important roles in directing intestinal epithelium cell migration and regulating proliferation as well [11].

Most sporadic colorectal cancers are initiated by activating Wnt pathway mutations and characterized by the stabilization of β-catenin. Although Wnt signaling remains constitutively active, most human colorectal cancers lose expression of EphB at the adenoma-carcinoma transition. Loss of EphB expression strongly correlates with degree of malignancy [15]. EphB4 is frequently downregulated in colorectal tumors through the aberrant hypermethylation of its promoter; patients with low EphB4 tumor levels had significantly shorter survival than patients in the high EphB4 group [16].

In the present study, we analyzed expression levels of EphB1 transcript and protein in a series of colorectal cancer and matched normal mucosa. Our data showed that expression of EphB1 was markedly varied among the colorectal cancer specimens. Downregulation of EphB1 transcript and protein was found in 34.6% (27/78) and 56.5% (39/69) of patients with colorectal cancer, respectively. Although expression of the EphB1 transcript was not completely consistent with expression of the EphB1 protein, either expression of EphB1 transcript or protein is significantly correlated with tumor pathological classification. EphB1 underexpression is often found in colorectal patients with poor differentiation and mucinous tumors. Our results indicate that EphB1 may have roles in differentiation of colorectal cells. Furthermore, cancer cells with low level of EphB1 protein show more invasive power. This can be interpreted as follows: by interacting with ligands of ephrins, EphB suppresses colorectal cancer progression by compartmentalization of tumor cells [10]. Loss of EphB1 protein expression can include EphB1 mutation, increased protein degradation,



Color version available online

Fig. 3. a Expression of EphB1 in plasma and cellular membrane of colorectal cancer cells. **b, c** The specificity of EphB1 antibody was investigated in colorectal cancer section by using blocking peptide. EphB1 signal is blocked in **c** when a serial section of **b** is probed with primary antibody premixed with antigen.

a defect in the translation or chromosomal deletions. We are currently investigating these possibilities.

The expression pattern of immunoreactivity of EphB1 in normal mucosa is very similar to that of staining of EphB2 receptor in normal intestinal crypts, in which EphB2 could be observed in the progenitor cells at the bottom of crypts and in a decreasing gradient from the bottom to the luminal epithelium [17]. We postulate that *EphB1* has the same roles that *EphB3* and *EphB4* play in colorectal cancer. The functional study of *EphB1* in colorectal cancer will be involved in our next project. Immunostaining of EphB1 was observed in the cytoplasm or both the cell membrane and cytoplasm, and particularly in the golgiosome (fig. 2, 3a). The pattern of EphB1 expression in colorectal cancer cells was focal with considerable variation in the intensity of staining throughout the neoplastic cell population (fig. 2c). However, all colorectal adenomas displayed evidence for homogeneous expression of EphB1 (fig. 2b). This suggests that loss of EphB1 by a proportion of cells in the neoplastic population occurs at the transition from adenoma to cancer.

Hypermethylation of CpG island at promoter region is an important epigenetic mechanism for downregulation of a gene. The promoter region of *EphB1* contains a dense 5'CpG island. Promoter methylation analysis was performed. However, methylated DNA was not found in any sample. Our data suggest that there are other mechanisms for the loss of EphB1 expression or promoter methylation in other regulatory regions.

In summary, EphB1 expression is lost in colorectal tumors with poor differentiation and cancer cells with low level of EphB1 protein show more invasive power. Our data show that EphB1 may have important physiologic roles in the intestinal epithelium and may also have roles in the development and prognosis of colorectal cancer.

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Non-Hodgkin lymphoma and obesity: A pooled analysis from the InterLymph consortium

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Nutritional status is known to alter immune function, a suspected risk factor for non-Hodgkin lymphoma (NHL). To investigate whether long-term over, or under, nutrition is associated with NHL, self-reported anthropometric data on weight and height from over 10,000 cases of NHL and 16,000 controls were pooled across 18 case-control studies identified through the International Lymphoma Epidemiology Consortium. Study-specific odds ratios (OR) were estimated using logistic regression and combined using a random-effects model. Severe obesity, defined as BMI of 40 kg m^{-2} or more, was not associated with NHL overall (pooled OR = 1.00, 95% confidence interval (CI) 0.70–1.41) or the majority of NHL subtypes. An excess was however observed for diffuse large B-cell lymphoma (pooled OR = 1.80, 95% CI 1.24–2.62), although not all study-specific ORs were raised. Among the overweight (BMI 25–29.9 kg m^{-2}) and obese (BMI 30–39.9 kg m^{-2}), associations were elevated in some studies and decreased in others, while no association was observed among the underweight (BMI < 18.5 kg m^{-2}). There was little suggestion of increasing ORs for NHL or its subtypes with every 5 kg m^{-2} rise in BMI above 18.5 kg m^{-2}. BMI components height and weight were also examined, and the tallest men, but not women, were at marginally increased risk (pooled OR = 1.19, 95% CI 1.06–1.34). In summary, whilst we conclude that there is no evidence to support the hypothesis that obesity is a determinant of all types of NHL combined, the association between severe obesity and diffuse large B-cell lymphoma may warrant further investigation.

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Non-Hodgkin lymphomas (NHL) can arise following rare inherited disorders of the immune system, long-term immunosuppressive drug therapy and viral infections such as human immunodeficiency virus (HIV) and Epstein-Barr virus (EBV). In such instances, severe immunosuppression resulting from exposure usually leads to the development of specific NHL subtypes. For the majority of NHL however, the cause remains unknown but it is suspected that factors which affect the immune system are involved. In particular, it has been suggested that the degree of adiposity might be important since over (as well as under) nutrition can alter immune function.[1,2] However, while several epidemiological studies have reported associations between excess weight and NHL[3–15] the evidence is far from conclusive.[16–28] Here we present a pooled analysis of self-reported height and weight on over 10,000 NHL cases and 16,000 controls from 18 case-control studies identified through the International Lymphoma Epidemiology Consortium (InterLymph: http://epi.grants.cancer.gov/InterLymph/).

Material and methods

Through the InterLymph forum, 18 case-control studies of NHL with anthropometric data collected across 13 countries in parts of North America, Europe and Japan between 1983 and 2004 were identified. Study designs are briefly outlined in Table I, and more details are published elsewhere.[3,8,14,18,24,29–36] Cases were identified using rapid ascertainment techniques, while controls were randomly selected from population registers (8 studies), outpatient clinics (3 studies) or inpatients (7 studies) hospitalized for a variety of nonneoplastic conditions such as circulatory, digestive or respiratory problems, or with traumatic or nontraumatic orthopaedic conditions. The appropriate ethical committees' approval was granted for each study and informed consent was given by all participants.

NHL diagnoses were pathologically confirmed and subsequently coded to the World Health Organisation (WHO) classification[37] (15 studies), the REAL classification (the 1999–2002 Italian study), or Working Formulation (North Italy and UCSF). Cases with HIV were excluded. Diagnostic codes from the different studies were combined as previously described.[38] The analysis here considers specific B-cell subtypes of NHL (diffuse large B-cell lymphoma: ICDO3 codes 9679/3, 9680/3, 9684/3; follicular lymphoma: 9690/3, 9691/3, 9695/3, 9698/3; chronic lymphocytic leukaemia/small lymphocytic lymphoma: 9670/3, 9823/3; marginal zone lymphoma: 9689/3, 9699/3; mantle cell lymphoma: 9673/3; Burkitt lymphoma: 9687/3, 9826/3 and other unspecified B-cell lymphoma: 9671/3, 9728/3), and T-cell lymphomas as a whole (9700/3, 9701/3, 9702/3, 9705/3, 9708/3, 9709/3, 9714/3, 9716/3, 9717/3, 9718/3, 9719/3, 9729/3, 9827/3) as well as NHL in total (defined by the above ICDO3 codes and 9591/3, 9675/3, and 9727/3).

In all studies, information on anthropometrics, demographics, lifestyle, occupations and medical histories were collected by in-person or telephone interviews. For the purposes of the present analyses, anonymized data were provided and checked for inconsistencies before coding uniformly. Within each study, height in meters was categorized using sex-specific quintiles of the height distribution among controls, and data were then combined across studies to reflect the relative position, rather than the absolute value, of this variable. In the statistical analysis, the referent category for height was taken as the 3rd quintile, since this central group contains the median and has the narrowest range. Usual adult weight was requested in 10 studies (Nebraska, UCSF, SCALE and EpiLymph studies). Elsewhere different questions were used (weight at diagnosis/interview (HERPACC1, HERPACC2); 1 year (NCI-SEER, British Columbia, North Italy, Italy); 2 years (Mayo Phase 1); or 5 years (UK) prior to diagnosis/interview).

For the pooled analysis, body mass index (BMI) was computed by dividing weight in kilograms by the square of height in metres where each study's weight variable was considered at the closest time point prior to diagnosis/interview, or else the usual adult weight. BMI was grouped using the World Health Organisation categories of underweight (<18.5 kg m^{-2}), normal (18.5–24.99 kg m^{-2}), grade 1 overweight (25–29.99 kg m^{-2}), grade 2 obese (30–39.99 kg m^{-2}) and grade 3 obese (40 kg m^{-2} or more).[39] For a person 1.7 m (5′7″) tall, these cut-off points relate to weights of 53 kg (118 lb), 72 kg (159 lb), 87 kg (191 lb) and 116 kg (255 lb) respectively. Socioeconomic status was defined by the level of education attained, except in British Columbia and the UK where self-reported household income and a census-based household deprivation indicator were used respectively; and no socioeconomic status information was collected in the Japanese studies (HERPACC1 and 2).

Statistical analysis followed similar methods to those employed in previous InterLymph pooling projects.[40–44] Firstly, individual data were combined in an unconditional logistic regression model adjusting for study, age, sex and race. To test for between-study heterogeneity, this model was compared using the likelihood ratio test with the model that included an additional term for interaction between the anthropometric variable and a variable indexing the studies. Heterogeneity was assumed to be present when the likelihood ratio test yielded a p-value <0.05. This flexible approach utilizes all data and provides one statistic to test for heterogeneity. Where the likelihood ratio test was not statistically significant, the pooled adjusted OR and 95% CI computed from all individual data in an unconditional logistic regression model are presented.

Between-study heterogeneity was further examined among risk estimates at each category of the anthropometric variables. Study-specific odds ratios (OR) and 95% confidence intervals (CI) adjusted for sex, age and race were computed using unconditional logistic regression.[45] For each category of height or BMI, the study-specific ORs were combined using a random effects meta-analysis to produce a combined OR and corresponding 95% CI. The extent of heterogeneity for each category was indicated by Cochran's Q-statistic which was considered statistically significant when $p < 0.10$. The I^2-statistic was also reported to describe the percentage of total variation in the study-specific ORs which was due to heterogeneity.[46]

Since the ORs were diverse across studies, a variety of approaches were applied to explore heterogeneity.[47] To assess relative obesity within study populations rather than the absolute value, BMI was grouped into quintiles based on the control distributions within each study before combining the relative quintile groupings across studies; these analyses are not presented here since their findings were similar to those reported. Sensitivity analyses using various stratifications and subsets of data were also conducted.[48] Study-specific ORs were combined by continent, study design and time period (corresponding to the original lymphoma classification used) as well as by level of participation. Given that the study-specific associations with BMI were heterogeneous in all analyses, forest plots with ORs pooled by continent were judged to be the most informative. Pooled ORs stratified by study design are also presented.

Within studies, analyses were performed separately for men, women, Caucasian subjects and persons aged 18–65. The resulting study-specific ORs were combined in a random-effects meta-analysis to examine heterogeneity. Potential confounding factors, such as smoking, alcohol and socioeconomic status, were assessed by comparing study-specific regression models with and without the confounding factor using the likelihood ratio test. A factor was considered a confounder when the likelihood ratio test was significant and the adjusted OR changed by more than 10%. Continuous variables corresponding to 10 cm increases in height and 5 kg m^{-2} increases in BMI were created to assess trends. All analyses were conducted using Stata.[49]

TABLE I – CHARACTERISTICS OF CASE-CONTROL STUDIES INCLUDED IN THE POOLED ANALYSIS

Study	Location	Year of diagnosis	Age range	Cases (N = 10,453)	Participation (%)	Controls source (N = 16,507)	N	Participation (%)	Reference
NCI-SEER	Detroit, Michigan; Iowa; Los Angeles, California; Seattle, Washington, USA	1998–2001	20–70	527	76	<65 years RDD; 65+ years random selection from Centers for Medicare and Medicaid Services, stratified by study area, age, sex and race	468	52	8
Nebraska NHL study	Nebraska, USA	1999–2002	20–75	387	74	RDD, frequency matched by age and sex	535	78	14
Mayo Clinic Phase 1	Iowa, Wisconsin, Minnesota, USA	2002–2005	18+	499	66	Frequency matched by age, sex and county of residence	499	70	n/a
UCSF	San Francisco, USA	1988–1995	21–74	1,304	72	RDD, frequency matched by age, sex, and county of residence	2,402	78	3
British Columbia Study	Vancouver and Victoria, British Columbia, Canada	2000–2004	20–82	828	78	Random selection from Client Registry of the Ministry of Health, frequency matched by age, sex and region	848	46	36
UK	Yorkshire, Lancashire, South Lakeland and parts of Southwest England	1998–2003	16–69	833	70	Random selection from general practice lists, individually matched by age, sex and region of residence	1,141	69	29
SCALE	Denmark and Sweden	2000–2002	18–74	3,055	81	Random selection from population register, frequency matched by sex and age	3,187	71	24
EpiLymph Ireland	Six hospitals on the East Coast of the Republic of Ireland	2001–2003	18–80	135	90	Hospital controls matched by age (±5 years), sex and study region	208	75	30
EpiLymph Finland	Finland	2001–2003	18–80	87		Hospital controls matched by age (±5 years), sex and study region	75		n/a
EpiLymph Germany	Ludwigshafen/Upper Palatinate, Heidelberg/Rhine-Neckar County, Würzburg/Lower Frankonia, Hamburg, Bielefeld and Munich	1999–2002	18–80	496	88	Random selection from population register, individually matched by sex, age and study region	710	44	31
EpiLymph France	Amiens, Dijon and Montpellier	2000–2003	18–80	206	91	Hospital controls matched by age (±5 years), sex and study region	276	74	30
EpiLymph Czech Republic	1 centre in Czech Republic	2001–2003	18–80	195	90	Hospital controls individually matched by age (±5 years), sex and study region	304	60	30
EpiLymph Spain	Barcelona, Tortosa, Reus and Madrid	1998–2002	18–80	428	82	Hospital controls matched by age (±5 years), sex and study region	631	96	32
EpiLymph Italy	2 centres in Italy	1998–2004	18–80	222	93	Random selection from population census list, matched by age (±5 years), sex and study region	336	66	30
Northern Italy	Aviano & Milan	1983–1992	17–79	429	>97	Patients admitted for acute, nonneoplastic, nonimmunologic conditions in the hospitals where cases diagnosed	1,157	>97	18
Italy	Aviano & Naples	1999–2002	18–84	225	97	Hospital controls, frequency matched by age (in 5-year bands), sex and study centre to cases of lymphohematopoietic neoplasms including NHL and hepatocellular carcinoma	504	91	33
HERPACC1	Aichi Cancer Centre, Nagoya, Japan	1988–2000	18–79	416	≈99	Random sample of patients not diagnosed with cancer, individually matched by age and sex	2,260	≈99	34,35
HERPACC2	Aichi Cancer Centre, Nagoya, Japan	2001–2004	18–79	181	≈99	Random sample of patients not diagnosed with cancer, individually matched by age and sex	966	≈99	35

Results

The pooled dataset from the 18 case-control studies comprised anthropometric information from 10,453 cases of NHL and 16,507 controls. Most cases (85%) were diagnosed with a B-cell lymphoma, 5% with a T-cell lymphoma and for 11%, immunophenotype was not known. The 3 most common NHL subtypes were diffuse large B-cell lymphoma (DLBCL) (32%), follicular lymphoma (FL) (22%) and chronic lymphocytic lymphoma/small lymphocytic lymphoma (CLL/SLL) (16%). A slightly higher proportion of cases were men (57%), 90% of all cases were Caucasian and the median age was 60 years. Cases tended to be older in age, of white race and of lower socioeconomic status than controls (data not shown).

Height distributions among male and female controls varied by study; for both sexes, the median height was highest in the American studies, generally decreased from Northern to Southern Europe, and was lowest in the two Japanese studies (data not shown). Among men, compared to the third quintile the odds ratio was increased in the highest quintile (OR = 1.19, 95% CI 1.06–1.34), but was close to one in the lowest two quintiles (Supplementary Table I). When examining trend within studies, no consistent population pattern emerged; most studies showed no evidence of a trend with 10 cm increases in height, 6 a significant positive trend and 2 a significant negative trend (data not shown). Similar patterns were observed for the majority of NHL subtypes. Little association between height and NHL, or its subtypes, was observed among women (Supplementary Table I).

Figure 1 gives the distribution of BMI among controls by study. Like height, studies conducted in the US had the greatest median BMI, and Japan the lowest. When BMI was classified using WHO categories, associations between BMI and NHL were heterogeneous between studies (likelihood ratio test: $\chi^2 = 139.1$, $p < 0.0001$). Study-specific ORs showed that the heterogeneity was most marked in Grade 1 overweight, where ORs ranged from 0.50 (95% CI 0.34–0.74) in EpiLymph Italy to 1.70 (95% CI 1.02–2.84) in EpiLymph Ireland and Grade 2 obese (ranging from OR = 0.42, 95% CI 0.24–0.74 in EpiLymph Italy to OR = 1.78, 95% CI 1.36–2.32 in UCSF) (Figs. 2*b* and 2c). In the underweight and Grade 3 obese categories, where the numbers of subjects were small, ORs were also diverse (ranging from OR = 0.27, 95% CI 0.03–2.34 in EpiLymph Ireland to OR = 3.14, 95% CI 0.41–23.9 in EpiLymph Finland; and from OR = 0.19, 95% CI 0.02–1.58 in EpiLymph Germany to OR = 4.23, 95% CI 1.51–11.9 in UK, respectively) (Figs. 2*a* and 2*d*). Trends with a 5 kg m^{-2} increase in BMI above 18.5 kg m^{-2} were significantly increased in 2 studies, significantly decreased in 4 studies and showed little effect in the remaining studies (Fig. 3). ORs were pooled across North America, Northern Europe, Southern Europe and Japan. In North America, a homogeneous increased OR was suggested for Grade 1 overweight (Fig. 2*b*) but no effect was found among Grade 3 obese (Fig. 2*d*), and with the exception of the Californian study (UCSF), no significant positive trends were observed (Fig. 3). Heterogeneity was still evident when the analyses were restricted to population-based studies conducted in the period 1998–2005; to those designed to code to the WHO classification; or to those where control participation rates were 70% or more. Similarly study-specific ORs were heterogeneous among men or women; subjects aged 18–65; or Caucasian subjects (data not shown).

Statistically significant between-study heterogeneity was also present for the 3 most common NHL subtypes (likelihood ratio tests for WHO BMI and DLBCL: $\chi^2 = 104.2$, $p = 0.002$; FL: $\chi^2 = 82.7$, $p = 0.003$; CLL/SLL: $\chi^2 = 58.7$, $p = 0.04$). For these three subtypes, as for NHL as a whole, study-specific ORs varied around one in all WHO BMI groups, with tests for heterogeneity in the two-stage random effects model being significant among Grade 1 overweight and Grade 2 obese (DLBCL: Supplementary Figures 1*a*–1*d*; FL: Supplementary Figures 3*a*–3*d*; CLL/SLL: Supplementary Figures 4*a*–4*d*. In the underweight and Grade 3 obese groups, the meta-analyses generally suggested that ORs were more homogeneous and the combined risk estimates were not significantly different from one. The pooled OR for DLBCL among Grade 3 obese was increased (OR = 1.80, 95% CI 1.24–2.62, $Q = 16.7$, $p = 0.40$, $I^2 = 4.4\%$), being elevated in North America and Northern Europe, but as with all analyses in this BMI group, study-specific risk estimates were diverse, based on small numbers of subjects, and with wide and overlapping confidence intervals (Supplementary Fig. 1*d*). Like NHL as a whole, a 5 kg m^{-2} increase in BMI did not consistently increase the risk of DLBCL (Supplementary Fig. 2) or the other subtypes (data not shown). ORs for the rarer B-cell lymphomas and T-cell lymphoma were mostly not significantly different between studies, probably due to the small number of cases, and there was little suggestion of associations between these NHL subtypes and BMI (Supplementary Table II).

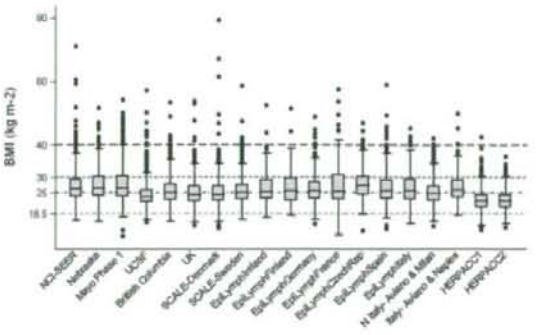

FIGURE 1 – Box-Whisker plot of body mass index among controls by study. Body mass index considered to be: Underweight if <18.5 kg m^{-2}; Normal weight-for-height if 18.5–24.99 kg m^{-2}; Grade 1 Overweight if 25–29.99 kg m^{-2}; Grade 2 Obese if 30–39.99 kg m^{-2}; and Grade 3 Obese if ≥40 kg m^{-2} Ref. 39.

Pooling data from studies with the highest WHO BMI prevalences of overweight/obese controls (EpiLymph Czech Republic, Nebraska, Mayo Phase 1, EpiLymph Italy, EpiLymph Germany, Italy-Aviano and Naples, and EpiLymph Finland) gave more homogeneous ORs (likelihood ratio test: $\chi^2 = 32.3$, $p = 0.12$). Within this subset of seven studies, there was still little evidence that higher than average BMI increases the risk of NHL and its subtypes (Table II). These findings were consistent when data were stratified by sex, age or race.

Discussion

The present InterLymph analysis, which is based on 18 studies from 13 countries, found little evidence to support the hypothesis that excess weight-for-height is associated with NHL. A slightly increased OR amongst the tallest men was observed compared to those who were of mid-range height but no association was found among women. The large number of subjects included in this analysis enabled examination of risks for subtypes of NHL. While findings for most were consistent with total NHL, an increased risk for DLBCL among persons with a BMI of 40 kg m^{-2} or more was observed in a meta-analysis of study-specific ORs. For DLBCL, ORs were elevated with overweight/obesity in North America and amongst the most obese in Northern Europe, yet studies in either region did not show an increasing trend with a 5 kg m^{-2} rise in BMI. Marked heterogeneity between studies was present for all categories of BMI, which remained when studies were combined by continent, study design and time period, WHO lymphoma classification used; and when data were restricted to men or women, persons aged 18–65, Caucasians alone or studies with participation rates of 70% or more. ORs were less heterogeneous amongst studies with the greatest proportions of controls

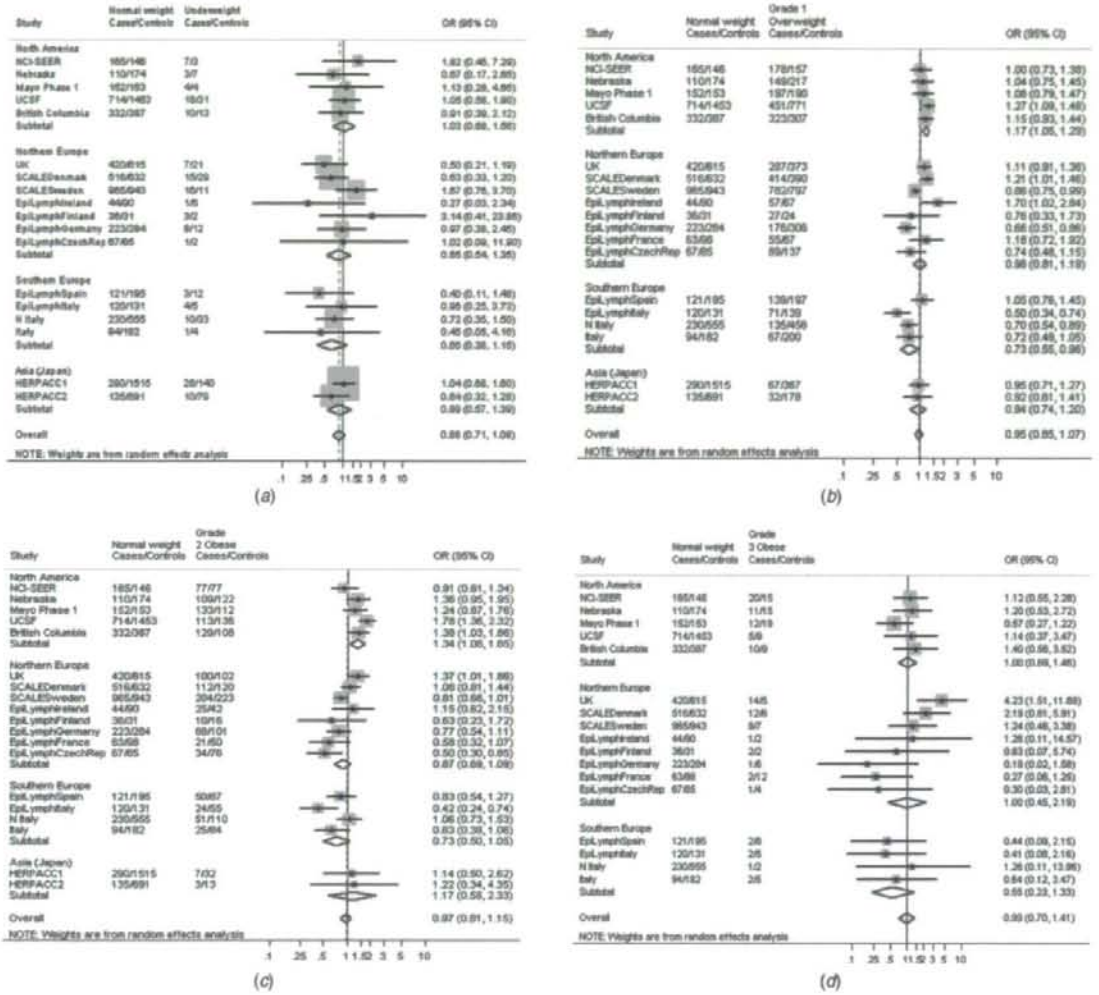

FIGURE 2 – (*a*) Meta-analysis of the risk of NHL associated with BMI <18.5 kg m^{-2} (Underweight) compared to BMI 18.5–24.99 kg m^{-2} (Normal weight). Overall test for heterogeneity: $Q = 13.0$, $p = 0.73$; Variation in odds ratios (OR) attributable to heterogeneity: $I^2 = 0.0\%$. For continents: North America: $Q = 1.04$, $p = 0.90$, $I^2 = 0.0\%$; Northern Europe: $Q = 7.87$, $p = 0.25$, $I^2 = 23.7\%$; Southern Europe: $Q = 1.03$, $p = 0.80$, $I^2 = 0.0\%$; Asia (Japan): $Q = 1.38$, $p = 0.24$, $I^2 = 27.5\%$. Test for heterogeneity between continents: $Q = 1.82$, $p = 0.61$. Pooled odds ratios by study design were: Population-based studies: OR = 0.91, 95% CI 0.68–1.21, $Q = 6.75$, $p = 0.56$, $I^2 = 0.0\%$; Clinic-based studies: OR = 0.92, 95% CI 0.65–1.31, $Q = 1.47$, $p = 0.48$, $I^2 = 0.0\%$; Hospital-based studies: OR = 0.67, 95% CI 0.39–1.17, $Q = 3.79$, $p = 0.58$, $I^2 = 0.0\%$. Test for heterogeneity between study designs: $Q = 1.04$, $p = 0.59$. (*b*) Meta-analysis of the risk of NHL associated with BMI 25–29.99 kg m^{-2} (Grade 1 overweight) compared to BMI 18.5–24.99 kg m^{-2} (Normal weight). Overall test for heterogeneity: $Q = 60.0$, $p < 0.001$; Variation in odds ratios (OR) attributable to heterogeneity: $I^2 = 70.0\%$. For continents: North America: $Q = 2.76$, $p = 0.60$, $I^2 = 0.0\%$; Northern Europe: $Q = 25.0$, $p = 0.001$, $I^2 = 72.1\%$; Southern Europe: $Q = 8.59$, $p = 0.04$, $I^2 = 65.1\%$; Asia (Japan): $Q = 0.02$, $p = 0.90$, $I^2 = 0.0\%$. Test for heterogeneity between continents: $Q = 23.4$, $p < 0.001$. Pooled odds ratios by study design were: Population-based studies: OR = 0.97, 95% CI 0.82–1.14, $Q = 41.6$, $p < 0.001$, $I^2 = 80.8\%$; Clinic-based studies: OR = 0.99, 95% CI 0.82–1.20, $Q = 0.44$, $p = 0.80$, $I^2 = 0.0\%$; Hospital-based studies: OR = 0.91, 95% CI 0.72–1.16, $Q = 14.0$, $p = 0.03$, $I^2 = 57.1\%$. Test for heterogeneity between study designs: $Q = 3.93$, $p = 0.14$. (*c*) Meta-analysis of the risk of NHL associated with BMI 30–39.99 kg m^{-2} (Grade 2 obese) compared to BMI 18.5–24.99 kg m^{-2} (Normal weight). Overall test for heterogeneity: $Q = 59.7$, $p < 0.001$; Variation in odds ratios (OR) attributable to heterogeneity: $I^2 = 69.8\%$. For continents: North America: $Q = 8.18$, $p = 0.08$, $I^2 = 51.1\%$; Northern Europe: $Q = 18.1$, $p = 0.01$, $I^2 = 61.2\%$; Southern Europe: $Q = 7.88$, $p = 0.05$, $I^2 = 62.0\%$; Asia (Japan): $Q = 0.01$, $p = 0.93$, $I^2 = 0.0\%$. Test for heterogeneity between continents: $Q = 25.4$, $p < 0.001$. Pooled odds ratios by study design were: Population-based studies: OR = 1.06, 95% CI 0.83–1.34, $Q = 41.3$, $p < 0.001$, $I^2 = 80.7\%$; Clinic-based studies: OR = 1.22, 95% CI 0.90–1.67, $Q = 0.03$, $p = 0.99$, $I^2 = 0.0\%$; Hospital-based studies: OR = 0.77, 95% CI 0.60–0.98, $Q = 8.51$, $p = 0.20$, $I^2 = 29.5\%$. Test for heterogeneity between study designs: $Q = 9.81$, $p = 0.007$. (*d*) Meta-analysis of the risk of NHL associated with BMI ≥40 kg m^{-2} (Grade 3 obese) compared to BMI 18.5–24.99 kg m^{-2} (Normal weight). Overall test for heterogeneity: $Q = 21.9$, $p = 0.15$; Variation in odds ratios (OR) attributable to heterogeneity: $I^2 = 26.8\%$. For continents: North America: $Q = 2.89$, $p = 0.58$, $I^2 = 0.0\%$; Northern Europe: $Q = 15.3$, $p = 0.03$, $I^2 = 54.4\%$; Southern Europe: $Q = 0.69$, $p = 0.88$, $I^2 = 0.0\%$. Test for heterogeneity between continents: $Q = 2.91$, $p = 0.23$. Pooled odds ratios by study design were: Population-based studies: OR = 1.33, 95% CI 0.88–2.00, $Q = 11.4$, $p = 0.18$, $I^2 = 29.7\%$; Clinic-based studies: OR = 0.57, 95% CI 0.26–1.22, No test for heterogeneity as only 1 study; Hospital-based studies: OR = 0.51, 95% CI 0.25–1.05, $Q = 2.07$, $p = 0.91$, $I^2 = 0.0\%$. Test for heterogeneity between study designs: $Q = 8.41$, $p = 0.015$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

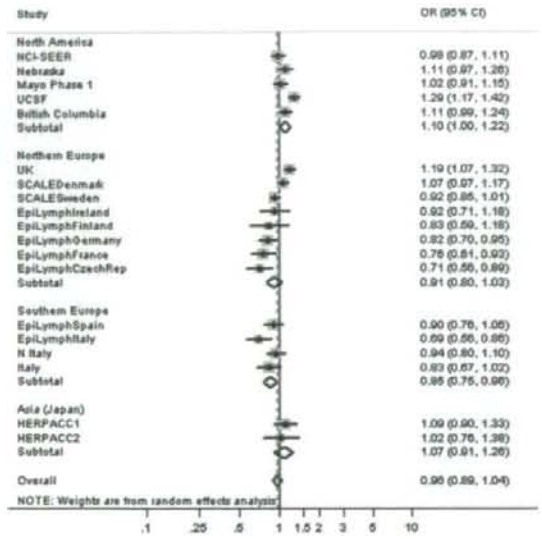

FIGURE 3 – Meta-analysis of the risk of NHL associated with 5 kg m^{-2} increase in BMI above 18.5 kg m^{-2} (Normal weight and above). Overall test for heterogeneity: $Q = 87.5$, $p < 0.001$; Variation in odds ratios (OR) attributable to heterogeneity: $I^2 = 79.4\%$. For continents: North America: $Q = 15.5$, $p = 0.004$, $I^2 = 74.1\%$; Northern Europe: $Q = 37.4$, $p < 0.001$, $I^2 = 81.3\%$; Southern Europe: $Q = 5.32$; $p = 0.15$; $I^2 = 43.6\%$; Asia (Japan): $Q = 0.12$, $p = 0.73$, $I^2 = 0.0\%$. Test for heterogeneity between continents: $Q = 29.0$, $p < 0.001$. Pooled odds ratios by study design were: Population-based studies: OR = 1.02, 95% CI 0.92–1.13, $Q = 57.7$, $p < 0.001$, $I^2 = 86.1\%$; Clinic-based studies: OR = 1.04, 95% CI 0.94–1.14, $Q = 0.34$, $p = 0.84$, $I^2 = 0.0\%$; Hospital-based studies: OR = 0.85, 95% CI 0.79–0.92, $Q = 6.09$, $p = 0.41$, $I^2 = 1.4\%$. Test for heterogeneity between study designs: $Q = 23.4$, $p < 0.001$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

with a BMI of 25 kg m^{-2} or more. Of the seven studies in this subset, no effect of BMI on NHL risk was observed, and the lack of association with obesity was consistent across NHL subtypes, amongst men and women, and at age ≤45, 46–55, 56–65 and ≥66 years.

Six of the case-control studies included in this pooled analysis have previously published data on NHL and obesity[3,8,11,14,23,24,50] and a further 12 are included here for the first time. Apart from case-control studies, adiposity has been investigated in cohorts where height and weight were measured[9,10,12,13,25–27] or self-reported,[5,15,20,21] and among persons with a hospital discharge for obesity.[4,19,22] Cohort studies have the advantage of prospectively collected information, although not necessarily at a relevant time point. Positive associations with obesity have been reported for some cohorts,[5,9,10,12,13,15] but not for others[4,19–22,25–27]; and a further case-control investigation nested within a cohort reported a reduced risk based on measured height and weight.[16] Only one additional study of case-control design—which is not part of the InterLymph consortium—has published its findings, observing an excess risk of NHL with obesity.[6]

Hitherto only a few individual case-control studies and two cohort studies have considered lymphoma subtypes, proposing an association with excess adiposity for DLBCL, but less so for FL and CLL/SLL.[8,11,14,15,21,22,24,50] A recent meta-analysis of published risk estimates suggested a slight increased risk of NHL, particularly DLBCL based upon data from both case-control and cohort studies.[51] The pooled analysis presented here has the advantage of being less susceptible to positive publication bias since it is based on all studies within the InterLymph consortium

TABLE II – NUMBER OF CASES AND CONTROLS, POOLED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR BODY MASS INDEX BY ALL NHL SUBTYPES AND THE THREE MOST COMMON NHL SUBTYPES IN STUDIES WITH THE HIGHEST PREVALENCE OF OVERWEIGHT/OBESE CONTROLS[1]

BMI[2]	Controls ($N = 2,963$)	NHL[3] ($N = 2,108$)	OR[4]	95% CI	DLBCL[3] ($N = 659$)	OR[4]	95% CI	FL[3] ($N = 457$)	OR[4]	95% CI	CLL/SLL[3] ($N = 381$)	OR[4]	95% CI
WHO category (kg m^{-2})													
<18.5	36	24	0.85	0.50–1.44	8	1.09	0.48–2.49	5	0.96	0.36–2.59	3	1.47	0.40–5.42
18.5–24.99	1,040	802	1	–	273	1	–	182	1	–	121	1	–
25–29.99	1,213	776	0.79	0.69–0.90	222	0.68	0.56–0.84	149	0.71	0.56–0.91	163	0.96	0.74–1.25
30–39.99	566	403	0.84	0.72–0.99	111	0.72	0.56–0.93	100	0.96	0.73–1.26	85	1.09	0.80–1.49
≥40	56	31	0.63	0.40–0.99	14	0.94	0.51–1.72	5	0.54	0.21–1.40	2	0.37	0.09–1.61
Missing	52	72			31			16			7		
Test for heterogeneity[5]			$\chi^2 = 32.2$	$p = 0.12$		$\chi^2 = 25.6$	$p = 0.27$		$\chi^2 = 24.7$	$p = 0.05$		$\chi^2 = 17.8$	$p = 0.16$

[1]Studies with highest prevalence of overweight/obese controls were EpiLymph Czech Republic, Nebraska, Mayo Phase 1, EpiLymph Italy, EpiLymph Germany, Italy-Aviano and Naples, and EpiLymph Finland.–[2]Body mass index grouped using WHO categories where <18.5 kg m^{-2} is considered Underweight; 18.5–24.99 kg m^{-2} Normal weight; 25–29.99 kg m^{-2} Grade 1 Overweight; 30–39.99 kg m^{-2} Grade 2 Obese; and ≥40 kg m^{-2} Grade 3 Obese.–[3]NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; CLL/SLL, chronic lymphocytic leukaemia/small lymphocytic lymphoma.–[4]Odds ratios and 95% confidence intervals adjusted for study, sex, age and race were estimated using unconditional logistic regression.–[5]Test for heterogeneity was conducted by testing for evidence of interaction between BMI and studies using the likelihood ratio test.