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A shared susceptibility locus in *PLCE1* at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma

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Abstract

We conducted a genome-wide association study of gastric cancer (GC) and esophageal squamous cell carcinoma (ESCC) in ethnic Chinese subjects in which we genotyped 551,152 single nucleotide polymorphisms (SNPs). We report a combined analysis of 2,240 GC cases, 2,115 ESCC cases, and 3,302 controls drawn from five studies. In logistic regression models adjusted for age, sex, and study, multiple variants at 10q23 had genome-wide significance for GC and ESCC independently. A notable signal was rs2274223, a nonsynonymous SNP located in *PLCE1*, for GC

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AUTHOR CONTRIBUTIONS C.C.A., N.D.F., N.H., Z.W., K.Y., X.-O.S., J.-M.Y., W.Z., L.M.D., W.-H.C., A.M.G., S.J.C. and P.R.T. organized and designed the study and served as the study steering committee. M.Y., J.Y., A.H., K.B.J., L.B., M.A.T. and S.J.C. supervised genotyping of samples. C.C.A., N.D.F., Z.W., K.Y., W.W., M.H.G., W.-H.C., A.M.G., S.J.C. and P.R.T. contributed to the design and execution of statistical analysis. C.C.A., Z.W. and S.J.C. wrote the first draft of the manuscript. C.C.A., N.H., X.-O.S., J.-M.Y., W.Z., S.M.D., M.P.L., T.D., Y.-L.Q., Y.-T. G., W.-P.K., Y.-B.X., Z.-Z.T., J.-H.F., C.W., C.A.G., J.F.F., W.-H.C., A.M.G. and P.R.T. conducted the epidemiologic studies and contributed samples to the GWAS and/or second phase. All authors contributed to writing the manuscript.

($P=8.40 \times 10^{-9}$; per allele odds ratio (OR) = 1.31) and ESCC ($P=3.85 \times 10^{-9}$; OR = 1.34). The association with GC differed by anatomic subsite. For tumors located in the cardia the association was stronger ($P=4.19 \times 10^{-15}$; OR= 1.57) and for those located in the noncardia stomach it was absent ($P=0.44$; OR=1.05). Our findings at 10q23 could provide insight into the high incidence rates of both cancers in China.

Gastric cancer and esophageal cancer cause more than 700,000 and 400,000 deaths respectively each year, and represent the 2nd and 6th leading causes of cancer death worldwide. For GC, infection with *Helicobacter pylori* is the primary etiologic factor in all populations, although the majority of infected individuals do not develop cancer. Smoking tobacco and drinking alcoholic beverages explain nearly 90% of ESCC cases in the United States and other Western countries, but these exposures represent minor factors in high-risk populations in China and elsewhere. Risk factors for ESCC in populations with high incidence rates include family history and dietary deficiencies, but a large proportion of the etiology in these populations remains unexplained. GC and ESCC occur in the Taihang Mountains of North-Central China at some of the highest rates reported for any cancer; over 20% of all deaths in this area have been attributed to these cancers, . However, the causes of the high rates and of the geographic correlation of these two anatomically adjacent but histologically distinct tumors have not been determined. The gastric cancers in this area occur primarily in the uppermost portion of the stomach (proximal 3 cm) and are referred to as gastric cardia cancers, while those in the remainder of the stomach are referred to as gastric noncardia cancers. In most other parts of China gastric noncardia cancers are the predominant upper gastrointestinal tract tumors.

To investigate the genetic contribution to these highly fatal diseases in ethnic Chinese subjects, we conducted parallel genome-wide association studies (GWAS) for GC and ESCC with shared controls. Using the Illumina 660W Quad chip, we scanned 4,987 samples from the case-control and case-only components of the Shanxi Upper Gastrointestinal Cancer Genetics Project (Shanxi) and 1,389 samples from a prospective cohort, the Linxian Nutrition Intervention Trials (NIT); both studies were conducted in the Taihang Mountains (Supplementary Table 1). After quality control metrics were applied (**Online Methods**), 551,152 SNPs were analyzed in 1,625 cases of GC, 1,898 cases of ESCC and 2,100 controls. 12,000 SNPs with minimal linkage disequilibrium (pair-wise $r^2 < 0.004$) were used to test for differences in population substructure and did not demonstrate significant evidence for population substructure within study (data not shown). In a second phase, we optimized TaqMan assays to genotype eight SNPs that were significant in the genome-wide phase for GC, ESCC, or both in an independent set of subjects (615 GC, 217 ESCC and 1202 controls) from the Shanxi and NIT studies and three additional prospective cohorts (The Shanghai Men's Health Study, the Shanghai Women's Health Study, and the Singapore Chinese Cohort Study) (Supplementary Table 1). For these eight SNPs, we conducted a combined analysis of 2,240 GC cases, 2,115 ESCC cases, and 3,302 controls (details in Supplementary Table 1).

The results of the initial GWAS for GC and ESCC, which were analyzed independently, are presented as Manhattan plots in Supplementary Figure 1 using P -values from 1 df trend tests

in logistic regression models adjusted for age, sex, and study. We found independent genome-wide significant associations at chromosome 10q23 for both GC and ESCC (Tables 1 and , Fig. 1). For GC, an association initially observed at chromosome 1q22 was not supported in the combined data (Table 1); additional studies are required to determine if this locus contributes to risk for GC in ethnic Chinese.

At 10q23, we analyzed a set of five correlated SNPs in both GC and ESCC, including two nonsynonymous variants. The strongest association for GC was with rs3781264 ($P = 3.76 \times 10^{-9}$; per allele OR = 1.36, 95% c.i. 1.23–1.50). The other four SNPs at 10q23 also showed genome-wide significance (Table 1). The associations differed when gastric cancers were divided into the two anatomic subsites. The strongest association for gastric cardia cancer was rs2274223 ($P = 4.19 \times 10^{-15}$; OR = 1.57, 95% c.i. 1.40–1.76), but there was no association for gastric noncardia cancers ($P = 0.44$; OR = 1.05, 95% c.i. 0.93 – 1.20). rs2274223 and other SNPs at 10q23 also showed genome-wide significance with ESCC ($P = 3.85 \times 10^{-9}$; OR = 1.34, 95% c.i. 1.22–1.48) (Table 2). We found consistent results when comparing the two studies from the high incidence areas of the Taihang Mountains (Supplementary Table 2). The five SNPs at 10q23, which have strong pair-wise LD (r^2 from 0.62 to 0.98 in controls), map to the Phospholipase C ϵ 1 gene (*PLCE1*) that lies adjacent to the nucleolar complex associated 3 homolog gene (*NOC3L*) (Fig. 1).

The SNPs that showed significant associations for GC and ESCC at 10q23 in the *PLCE1* gene included two SNPs that result in missense mutations in the coding region, rs2274223 (Arg1927His) and rs3765524 (Ile1777Thr). Further work is required to determine if either of these SNPs is functionally important, but the findings suggest a single locus associated with risk for both cancers. Notably, when gastric cancers were divided into the two distinct anatomic locations, the association was restricted to tumors of the cardia (Table 1).

PLCE1 is a member of the phospholipase C family of proteins and, uniquely within this family, it interacts with the proto-oncogene *ras* among other proteins. Variants in *PLCE1* are known to cause early-onset nephrotic syndrome in humans, but this gene may also be linked to carcinogenic processes. *PLCE1* knockout mice are resistant to the promoting effects of 12-O-tetradecanoylphorbol-13-acetate in 7,12-dimethylbenzanthracene-induced skin carcinogenesis and are resistant to intestinal tumor formation when crossed with APC^{min/+} mice. In addition, the SNPs reside in an area between two recombination hot spots that also includes *NOC3L*, which has been linked to control of DNA replication during mitotic clonal expansion.

For ESCC, we initially observed an independent significant association with rs738722 at chromosome 22q12 ($P = 5.67 \times 10^{-8}$; OR = 1.32, 95% c.i. 1.19–1.45) (Table 2) in the first phase, but the association was not statistically significant in the second phase by itself. In the combined data the association remained strong ($P = 1.41 \times 10^{-8}$; OR = 1.30, 95% c.i. 1.19–1.43). This SNP maps to a region within the CHK2 checkpoint homolog gene (*CHEK2*), but is also in LD with regions of the Hsc B iron-sulfur cluster co-chaperone homolog gene (*HSCB*) (Supplementary Fig. 2). Previous studies of Caucasian populations have suggested an association between uncommon variants in *CHEK2* (rs2267130 and rs17879961) and risk of upper aerodigestive tract cancers, , but these SNPs were not included in our scan. Rare

variants in *CHEK2* have also been associated with susceptibility to breast, colorectal, and other cancers. This association appears promising, but with the lack of independent confirmation further studies are needed to validate it.

We also examined loci previously reported in a GWAS for GC (Supplementary Table 3). Specifically, we examined rs2920297 and rs2294008 at 8q24; both SNPs are in proximity to the prostate stem cell antigen gene (*PSCA*). We found no associations for GC, but when we restricted our analysis to gastric noncardia tumors, both SNPs showed associations of similar magnitude to those reported in a recent meta-analysis of East Asian studies (e.g. rs2294008 OR = 1.35, 95% c.i. 0.94–1.94). For ESCC, we also examined SNPs marking the alcohol-metabolizing genes *ADH1B* (rs1159918 and rs1042026) and *ALDH2* (rs3782886 and rs671) that have been reported in candidate gene studies and in a GWAS. Overall and in strata defined by alcohol drinking and tobacco smoking, we found no associations with these SNPs (Supplementary Table 4), perhaps due to the different environmental risk factors for ESCC in our study populations compared to previous studies with strong alcohol- and tobacco-related risks. In the Shanxi and NIT studies, the only two studies included in this portion of the analysis, alcoholic beverage and tobacco use are not major ESCC risk factors.

In summary, we conducted parallel genome-wide association studies for GC and ESCC in ethnic Chinese subjects. Variants at 10q23 in *PLCE1* showed genome-wide significant associations for gastric cardia cancer and ESCC. These findings suggest that a common genetic mechanism may contribute to the etiology of both cancers. Fine mapping and sequencing in these loci will be required to determine the optimal genetic variants to be studied in laboratory systems to explain these association signals. Additional studies are needed to confirm and discover more loci associated with risk for GC and ESCC in populations in East Asia and elsewhere.

ONLINE METHODS

Study participants

Study participants for the GWAS were drawn from two studies, the Shanxi Upper Gastrointestinal Cancer Genetics Project (Shanxi) and the Linxian Nutrition Intervention Trial (NIT), a prospective cohort. For the second phase, we genotyped additional subjects from Shanxi and NIT as well as subjects from the Shanghai Men's Health Study (SMHS), the Shanghai Women's Health Study (SMHS), and the Singapore Chinese Health Study (SCHS) (Supplementary Table 1). The Shanxi study controls were matched on age and sex for the case-control portion, while for the NIT controls were selected as a case-cohort and frequency matched on age and sex. For the SMHS, SWHS, and SCHS cohorts, controls were alive, free of upper gastrointestinal tract cancer, and matched to cases as described in Supplementary Table 1. For the Shanxi and NIT study, tumor anatomic location was known for all cases and >85% of cases had pathologic confirmation. For the three cohorts added in the second phase, the proportion with anatomic location in the stomach is given in Supplementary Table 1 and pathologic confirmation was available for >95% of cases. All examined esophageal cancers were squamous cell carcinomas (ESCC) and all examined gastric cancers (GC) were adenocarcinomas. Cardia cancers were located in the proximal 3 cm of the stomach, while noncardia cancers were those in the remainder of the stomach.

Gastric cancers without location information were included in total GC analyses but excluded from GC anatomic subsite analyses.

Each of the five participating studies obtained informed consent from subjects and from their studies Institutional Review Board(s). The NCI Special Studies-Institutional Review Board approved the overall GWAS study.

Genotyping and quality control

Genome-wide scanning was attempted on 6,384 samples using the Illumina 660W Quad chip. After excluding 8 samples with no observed intensity data, the 6,376 remaining samples were analyzed, including 4,987 from the Shanxi study and 1,389 from the NIT. Clustering was performed with 1,270 previously scanned Caucasian samples in order to improve calling for low MAF SNPs in the East Asian samples.

Participants were excluded because of: 1) completion rate lower than 94% (n=485 samples); 2) abnormal heterozygosity values of less than 25% or greater than 30% (n=53, among which 36 were also excluded for low completion rates); 3) discordant expected duplicates (n=3 pairs); 4) concordant unexpected duplicates (n=5 pairs, all from Shanxi); 5) gender discordance (n=55, all from Shanxi); 6) phenotype exclusions (due to ineligibility or incomplete information) (n=46). We checked for relatedness among study subjects using genotypes for all subject pairs with identity-by-state greater than 45%. These were input into GLU qc.ibds module (<http://code.google.com/p/glu-genetics/>) to estimate the identity-by-degree ratio and infer the degree of relatedness (1–2nd degree). We found 20 full sibling pairs, 2 parent-child pairs, and 22 half-sibling pairs. This level of relatedness was not surprising because of the geographic proximity of subjects accrued in the two studies. We selected and retained one from each of the 1st degree relative pair and excluded the other (n=22) for the PCA but included all for the association analyses. For the 132 known duplicate pairs the concordance was 99.98%.

Using 12,000 SNPs in low linkage disequilibrium (pair-wise $r^2 < 0.004$), we identified and excluded two subjects with less than 90% Asian ancestry based on STRUCTURE analysis (<http://pritch.bsd.uchicago.edu/structure.html>) (Supplementary Fig. 3). For the Shanxi and NIT study subjects passing the QC metrics, principal component analysis showed borderline significant differences between but not within studies. For subsequent analyses, we adjusted for study.

For all subjects in the genome-wide scan phase, we attempted 657,364 genotype assays. For analysis, we removed SNPs with a call rate <90%. 551,152 SNPs were advanced to the association analysis. Quantile-quantile plots (Supplementary Fig. 4) for case-control analyses were separately examined for GC and ESCC and there was no evidence for significant problems with population substructure or case-control matching: The unscaled λ for GC and ESCC were 0.990 and 0.989 respectively, while λ_{1000} for GC and ESCC were 0.995 and 0.994, respectively. The Illumina Infinium genotype probe cluster plots for select SNPs (rs2274223 and rs3781264) are shown in Supplementary Figure 5.

After completion of the genome-wide phase, we selected six SNPs at10q23, two at 1q22, and two at 22q12 for TaqMan genotyping in our second phase. All ten SNPs were at or near genome-wide significance for total GC, ESCC, or both. For the selected SNPs, we successfully optimized eight TaqMan assays (ABI), while two failed manufacturing or validation. For the second phase using TaqMan, we included samples from the Shanxi and NIT study that had not been scanned or failed QC metrics in the genome-wide phase as well as samples from three prospective cohort studies of subjects of Chinese ethnicity (SMHS, SWHS, and SCHS) (Supplementary Table 1). In total, we completed TaqMan assays on 2034 subjects. After standard quality control metrics were applied, the sample completion rate overall was 98.8%. Concordance between called Illumina genotypes and TaqMan was greater than 99.4%.

Association analysis

We used logistic regression models to estimate associations between genetic variants and disease risk. Primary models were adjusted for age in 10-year groups, sex, and study. We report trend models (Tables 1,), but also fit genotype models for comparison (Supplementary Table 3). All reported *P*-values are based on two-sided tests.

In the second and combined phases, logistic regression models were adjusted for age, sex, and study. Because previous studies reported an interaction between risk of ESCC, alcohol or tobacco consumption, and variants marking the *ADH1B* or *ALDH2* gene loci, we fit models both adjusted for and stratified on these factors (Supplementary Table 4).

Data analysis and management was performed with GLU (Genotyping Library and Utilities version 1.0), a suite of tools available as an open-source application for management, storage and analysis of GWAS data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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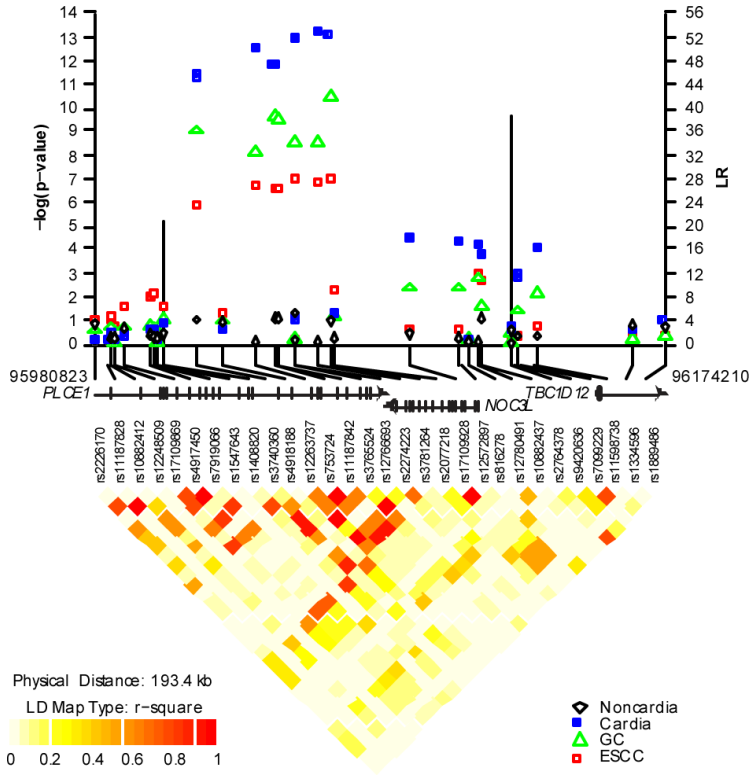


Figure 1. Association results, recombination, and linkage disequilibrium plots for the region of 10q23 with genome-wide significance for gastric cancer (GC) and esophageal squamous cell carcinoma (ESCC)

P-values were derived from 1 df trend tests in logistic regression models adjusted for age, sex, and study and are shown with the LR values for putative recombination hotspots using SequenceLDhot (vertical bars). Pair-wise r^2 are displayed at the bottom for all SNPs included in the GWAS analysis. Coordinates refer to genome Build 36.1. The figure depicts a region of chromosome 10q23 (95,980,823- 96,174,210) that includes the *PLCE1* gene and the different symbols indicate the four different endpoints of total GC, gastric cardia cancers, gastric noncardia cancers, or ESCC.

Table 1

Association between SNPs at 10q23 and 1q22 and risk for gastric cancer in all cases and by anatomic location within the stomach

Cancer group and chromosome location	NCBI dbSNP identifier (major; minor allele)	MAF Controls, Cases combined	Genome-wide phase		Second phase		Combined	
			P _{idf} score	OR (95%CI) per allele	P _{idf} score	OR (95%CI) per allele	P _{idf} score	OR (95%CI) per allele
Total Gastric				Controls/Cases 2100/1625		Controls/Cases 1202/615		Controls/Cases 3302/2240
10q23	rs2274223 (A, G)	0.209, 0.259	2.33 × 10 ⁻⁹	1.40 (1.25–1.56)	0.12	1.15 (0.97–1.36)	8.40 × 10 ⁻⁹	1.31 (1.19–1.43)
	rs3765524 (C, T)	0.207, 0.259	2.78 × 10 ⁻⁹	1.39 (1.25–1.56)	0.079	1.17 (0.98–1.38)	5.32 × 10 ⁻⁹	1.31 (1.20–1.44)
	rs3781264 (T, C)	0.152, 0.199	3.94 × 10 ⁻¹¹	1.51 (1.33–1.70)	0.40	1.09 (0.90–1.31)	3.76 × 10 ⁻⁹	1.36 (1.23–1.50)
	rs11187842 (C, T)	0.147, 0.190	3.65 × 10 ⁻¹⁰	1.48 (1.31–1.68)	0.42	1.08 (0.89–1.31)	2.53 × 10 ⁻⁸	1.34 (1.21–1.49)
	rs753724 (G, T)	0.147, 0.190	2.65 × 10 ⁻¹⁰	1.49 (1.31–1.68)	0.51	1.07 (0.88–1.30)	2.74 × 10 ⁻⁸	1.34 (1.21–1.49)
1q22	rs4072037 (A, G)	0.159, 0.125	1.10 × 10 ⁻⁶	0.71 (0.62–0.82)	0.083	0.84 (0.69–1.02)	4.22 × 10 ⁻⁷	0.75 (0.67–0.84)
	rs4460629 (C, T)	0.142, 0.112	3.37 × 10 ⁻⁷	0.68 (0.59–0.79)	0.34	0.91 (0.74–1.11)	2.26 × 10 ⁻⁶	0.75 (0.67–0.85)
Cardia				Controls/Cases 2100/1110		Controls/Cases 1202/103		Controls/Cases 3302/1213
10q23	rs2274223 (A, G)	0.209, 0.291	5.88 × 10 ⁻¹⁴	1.59 (1.41–1.80)	0.016	1.49 (1.08–2.07)	4.19 × 10 ⁻¹⁵	1.57 (1.40–1.76)
	rs3765524 (C, T)	0.207, 0.289	9.94 × 10 ⁻¹⁴	1.59 (1.40–1.79)	0.014	1.50 (1.08–2.08)	7.36 × 10 ⁻¹⁵	1.56 (1.40–1.75)
	rs3781264 (T, C)	0.152, 0.222	7.94 × 10 ⁻¹⁴	1.66 (1.45–1.90)	0.17	1.29 (0.89–1.87)	1.06 × 10 ⁻¹³	1.60 (1.41–1.81)
	rs11187842 (C, T)	0.147, 0.211	1.44 × 10 ⁻¹²	1.63 (1.42–1.87)	0.25	1.25 (0.86–1.81)	2.56 × 10 ⁻¹²	1.56 (1.38–1.77)
	rs753724 (G, T)	0.147, 0.210	1.61 × 10 ⁻¹²	1.63 (1.42–1.87)	0.49	1.15 (0.77–1.70)	5.21 × 10 ⁻¹²	1.56 (1.37–1.76)
1q22	rs4072037 (A, G)	0.159, 0.122	3.37 × 10 ⁻⁴	0.75 (0.65–0.88)	0.18	0.74 (0.48–1.15)	9.45 × 10 ⁻⁵	0.75 (0.65–0.87)
	rs4460629 (C, T)	0.142, 0.108	1.13 × 10 ⁻⁴	0.72 (0.61–0.85)	0.58	0.89 (0.58–1.36)	1.27 × 10 ⁻⁴	0.74 (0.64–0.86)
Noncardia				Controls/Cases 2100/515		Controls/Cases 1202/402		Controls/Cases 3302/917
10q23	rs2274223 (A, G)	0.209, 0.221	0.78	1.02 (0.86–1.22)	0.34	1.10 (0.90–1.35)	0.44	1.05 (0.93–1.20)
	rs3765524 (C, T)	0.207, 0.222	0.73	1.03 (0.87–1.22)	0.25	1.12 (0.92–1.37)	0.32	1.07 (0.94–1.21)
	rs3781264 (T, C)	0.152, 0.171	0.094	1.18 (0.97–1.42)	0.60	1.06 (0.85–1.33)	0.14	1.11 (0.97–1.29)
	rs11187842 (C, T)	0.147, 0.166	0.099	1.17 (0.97–1.42)	0.55	1.07 (0.86–1.34)	0.13	1.12 (0.97–1.29)
	rs753724 (G, T)	0.147, 0.167	0.070	1.19 (0.99–1.44)	0.53	1.08 (0.86–1.35)	0.098	1.13 (0.98–1.30)

Cancer group and chromosome location	NCBI dbSNP identifier (major, minor allele)	MAF Controls, Cases <i>combined</i>	Genome-wide phase		Second phase		Combined	
			$P_{idf\ score}$	OR (95%CI) per allele	$P_{idf\ score}$	OR (95%CI) per allele	$P_{idf\ score}$	OR (95%CI) per allele
1q22	rs4072037 (A, G)	0.159, 0.126	7.26×10^{-6}	0.60 (0.47-0.75)	0.40	0.91 (0.73-1.14)	5.74×10^{-5}	0.72 (0.62-0.85)
	rs4460629 (C, T)	0.142, 0.115	1.53×10^{-5}	0.59 (0.47-0.75)	0.79	0.97 (0.77-1.22)	5.38×10^{-4}	0.75 (0.64-0.88)

Minor allele frequencies were computed from the combined phase. Results were derived from logistic regression models using genotype trend tests adjusted for age (10 year categories), sex, and study. The total of 2,240 gastric cancer cases included all cardia and noncardia cancers plus 110 unclassified gastric cancers which were added in the second phase.

Table 2
Association between SNPs at 22q12 and 10q23 and risk for esophageal squamous cell carcinoma

Chromosome location	NCBI dbSNP identifier (major, minor allele)	MAF Controls, Cases combined	Genome-wide phase			Second phase			Combined	
			P _{1df} score	OR (95%CI) per allele	Controls/Cases	P _{1df} score	OR (95%CI) per allele	Controls/Cases	P _{1df} score	OR (95%CI) per allele
ESCC				Controls/Cases 2100/1898	Controls/Cases 1202/217					Controls/Cases 3302/2115
10q23	rs2274223 (A, G)	0.209, 0.259	1.19 × 10 ⁻⁷	1.33 (1.20-1.48)	2.13 × 10 ⁻⁴	1.59 (1.24-2.05)	3.85 × 10 ⁻⁹	1.34 (1.22-1.48)		
	rs3765524 (C, T)	0.207, 0.258	9.42 × 10 ⁻⁸	1.34 (1.20-1.49)	6.01 × 10 ⁻⁵	1.66 (1.29-2.12)	1.74 × 10 ⁻⁹	1.35 (1.22-1.49)		
	rs3781264 (T, C)	0.152, 0.194	1.15 × 10 ⁻⁷	1.38 (1.22-1.55)	7.92 × 10 ⁻⁴	1.60 (1.21-2.11)	7.30 × 10 ⁻⁹	1.38 (1.23-1.53)		
	rs11187842 (C, T)	0.147, 0.187	2.67 × 10 ⁻⁷	1.37 (1.21-1.54)	3.91 × 10 ⁻⁴	1.64 (1.25-2.17)	1.20 × 10 ⁻⁸	1.37 (1.23-1.53)		
	rs753724 (G, T)	0.147, 0.187	2.23 × 10 ⁻⁷	1.37 (1.22-1.55)	5.13 × 10 ⁻⁴	1.63 (1.24-2.16)	1.15 × 10 ⁻⁸	1.38 (1.23-1.54)		
22q12	rs738722 (C, T)	0.254, 0.308	5.67 × 10 ⁻⁸	1.32 (1.19-1.45)	0.14	1.20 (0.94-1.53)	1.41 × 10 ⁻⁸	1.30 (1.19-1.43)		

Minor allele frequencies were computed from the combined phase. Results were derived from logistic regression models using genotype trend tests adjusted for age (10 year categories), sex, and study.