Research Article

Cancer Epidemiology, Biomarkers & Prevention

A GWAS Meta-analysis and Replication Study Identifies a Novel Locus within *CLPTM1L/TERT* Associated with Nasopharyngeal Carcinoma in Individuals of Chinese Ancestry

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Abstract

Background: Genetic loci within the major histocompatibility complex (MHC) have been associated with nasopharyngeal carcinoma (NPC), an Epstein-Barr virus (EBV)-associated cancer, in several GWAS. Results outside this region have varied.

Methods: We conducted a meta-analysis of four NPC GWAS among Chinese individuals (2,152 cases; 3,740 controls). Forty-three noteworthy findings outside the MHC region were identified and targeted for replication in a pooled analysis of four independent case–control studies across three regions in Asia (4,716 cases; 5,379 controls). A meta-analysis that combined results from the initial GWA and replication studies was performed.

Results: In the combined meta-analysis, rs31489, located within the *CLPTM1L/TERT* region on chromosome 5p15.33, was strongly associated with NPC (OR = 0.81; *P* value 6.3×10^{-13}). Our results also provide support for associations reported from published NPC GWAS—rs6774494 ($P = 1.5 \times 10^{-12}$; located in the *MECOM* gene region), rs9510787 ($P = 5.0 \times 10^{-10}$; located in the *TNFRSF19* gene region), and rs1412829/rs4977756/rs1063192 ($P = 2.8 \times 10^{-8}$, $P = 7.0 \times 10^{-7}$, and $P = 8.4 \times 10^{-7}$, respectively; located in the *CDKN2A/B* gene region).

Conclusions: We have identified a novel association between genetic variation in the *CLPTM1L/TERT* region and NPC. Supporting our finding, rs31489 and other SNPs in this region have been reported to be associated with multiple cancer sites, candidate-based studies have reported associations between polymorphisms in this region and NPC, the *TERT* gene has been shown to be important for telomere maintenance and has been reported to be overexpressed in NPC, and an EBV protein expressed in NPC (LMP1) has been reported to modulate *TERT* expression/telomerase activity.

Impact: Our finding suggests that factors involved in telomere length maintenance are involved in NPC pathogenesis. *Cancer Epidemiol Biomarkers Prev; 25(1); 188–92. ©2015 AACR.*

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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doi: 10.1158/1055-9965.EPI-15-0144

Introduction

Nasopharyngeal carcinoma (NPC) is linked to Epstein-Barr virus (EBV) infection. While EBV infection is ubiquitous, NPC incidence varies considerably around the world (1). It is hypothesized that genetic differences across populations partly explain the predilection of this cancer to individuals of Southeast Asian descent. Several lines of evidence support a role for genetic susceptibility in NPC. The disease clusters within families (1). Also, numerous studies have implicated polymorphisms in candidate genes in NPC (2, 3). The most consistent evidence has been for an association between HLA and NPC, an association that is biologically plausible given the central role of EBV in NPC and of HLA in immune presentation/handling (4).

Several NPC GWAS have recently been published (5–9); all provided support for the importance of genetic factors and clearly confirmed the involvement of genes in the MHC region (region where *HLA* genes reside) in NPC. Associations outside the MHC were also reported from these GWAS but were not as strong or consistent, suggesting the need for pooling across studies and larger efforts to identify novel genes involved in this disease (5–9).

We report a meta-analysis of four published GWAS (2,152 cases; 3,740 controls), followed by replication of noteworthy findings in case–control studies from the populations where the GWAS were conducted (4,716 cases; 5,379 controls). Our analysis identified a novel association between *CLPTM1L/TERT* and NPC and confirmed reported associations for SNPs located in the *MECOM*, *TNFRSF19*, and *CDKN2A/2B* gene regions.

Materials and Methods

The GWAS contributing to our meta-analysis included histologically confirmed NPC cases and region-specific controls restricted to individuals of Chinese ethnicity (Table 1; refs. 5–8). Genotyping for these four GWAS was performed using Illumina platforms. The two Malaysian GWAS were analyzed as one. All data included passed QC filtering criteria as described for the respective studies (5–8).

To maximize coverage across studies, genome-wide imputations were performed for each study using typed SNPs. SNPs with call rates >90%, minor allele frequencies >3%, and that had genotype distributions that did not deviate from the expected by Hardy–Weinberg equilibrium (in controls; $P > 10^{-6}$) were retained for imputation using IMPUTE2. HapMap reference data were used (HapMap phase III, CHB+CHD+JPT data from IMPUTE2 website). Imputed genotypes with information score <90%, MAF <3%, or missing >10% were excluded. GTOOL (http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html) was used for data conversions.

For each GWAS, SNPs were analyzed by logistic regression under a log additive model, adjusting for age and cryptic population stratification. To define population stratification adjustment factors, principal component analysis was performed (EIGENSTRAT) using a pruned set of 30,956 SNPs defined based on pairwise linkage disequilibrium ($r^2 < 0.05$ among Chinese) and restricted to SNPs with MAF >3%. The top 10 eigenvectors were evaluated for their association with NPC (separately for each individual GWAS) and included in the final logistic models if *P*-value <0.05 by the Wald test.

Using results from individual GWAS, we identified the 500 SNPs with the lowest *P* values from each of the studies after exclusion of SNPs that could not be imputed or failed QC filtering. We combined these study-specific lists of top-SNPs into a single list for consideration as part of the present meta-analysis. In total, 1,590 SNPs were identified through this process and these 1,590 SNPs comprised the basis for the present meta-analysis. Summary statistics (number cases/controls, genotype counts, β coefficients, and SDs) were obtained from individual studies for selected SNPs and a meta-analysis was performed using a fixed effects model (R-program).

SNPs were selected for replication as follows. We arbitrarily ranked (by *P* value) the top 200 hits (all with *P* values <0.0167) from the meta-analysis for which the direction of the association was consistent across all individual studies. We then selected SNPs with *P* values $<1 \times 10^{-5}$ that were 250 kb+ from other selected SNPs. For SNPs within 250kb of another SNP on the list, we retained SNPs that had an $r^2 \leq 0.80$ (based on Chinese data from the 1 k genome project) and the SNP with the smaller *P* value when the r^2 between SNPs was >0.80. Thirty SNPs were selected based on these criteria. We added 14 SNPs nominated by consortium members based on results from individual GWAS and other information from candidate-based studies and other studies in the published literature. One SNP that qualified based on the criteria above but failed in the design of the custom array described below was excluded (rs11865086). A second SNP that qualified but failed in the custom array design (rs6931820) was replaced with a SNP in strong LD with the original SNP (rs1324103; $r^2 = 0.88$). In total, 43 SNPs were evaluated in the replication phase of our effort.

Replication studies were restricted to studies among individuals of Chinese descent. In total, we included 4,716 cases and 5,379 controls across four case–control studies in Mainland China, Malaysia, and Taiwan (Table 1). All four studies were hospital-based, recruiting NPC cases from selected hospitals in

Table 1. Summary of studies included in this analysis

Phase	Ref. #	Location	Genotyping platform	# Cases	# Controls
GWAS	6	Southern China	Illumina Hap610	1,583	2,979
GWAS	7	Taiwan	Illumina 600 Hap550v3_A BeadChips	277	285
GWAS	5	Malaysia (1)	Illumina Hap550v3 BeadChip	108	240
GWAS	8	Malaysia (2)	Illumina Human OmniExpress_12 v1.1 BeadChip	184	236
GWAS-SUM				2,152	3,740
Replication I		Southern China I	Sequenom custom array	3,525	4,121
Replication I		Malaysia	Sequenom custom array	335	405
Replication I		Taiwan I	Sequenom custom array	352	312
Replication I		Taiwan II	Sequenom custom array	504	541
Replication I - SUM				4,716	5,379
Total				6,868	9,119

their respective geographical area. For the southern China study, cases were recruited from the Sun Yat-Sen University Cancer Center (Guangzhou, China) and the Southern Medical University Hospital. For the Malaysia study, cases were recruited from the University of Malaysia Medical Center (Kuala Lumpur, Malaysia) and from a network of additional hospitals across the country. For the two Taiwan studies, cases were recruited form the National Taiwan University (Taipei, Taiwan) and MacKay Memorial hospitals and from the Chang Gung Memorial and Linkou hospitals, respectively. Cases were restricted to adults with histologically confirmed NPC. Geographically matched controls of Chinese descent were frequency (southern China, Malaysia, and Taiwan II studies) or individually (Taiwan I study) matched to cases on age and gender. Controls did not have a history of NPC diagnosis. Studies were reviewed/approved by ethical committees and informed consent was obtained from participants.

A custom designed array containing the 43 SNPs selected for replication was developed using the Sequenome MassARRAY iPlex assay (Supplementary Table S1). Testing was performed in one of two laboratories. To ensure comparable quality across laboratories, a common QC panel consisting of 94 HapMap samples was tested. Percent agreement across laboratories for the 43 SNPs tested was 97% (range: 82%–100%; agreement was >85% for all but two SNPs: rs189897 and rs4714505).

To analyze the replication studies, individual genotyping results were pooled and an additive logistic regression model used to evaluate the effect of each SNP, adjusting for study. To summarize information across the GWAS and replication studies,

Table 2. Results from GWAS meta-analysis and replication study for 43 SNPs selected for replication

								GWAS meta-analysis		Replication study		Combined	
SNP	Gene neighborhood	Chr	Location[a]	Selection criteria[b]	MAF (Ctrls)[c]	Major allele	Minor allele	OR	*P*	OR	*P*	OR	*P*
rs31489	CLPTM1L/TERT	5	1342714	2	0.22	C	A	0.85	1.8E-03	0.79	4.3E-11	0.81	6.3E-13
rs6774494	MECOM	3	169082633	2	0.36	A	G	0.81	4.0E-07	0.86	3.4E-07	0.84	1.5E-12
rs9510787	TNFRSF19	13	24205195	2	0.35	A	G	1.2	1.9E-05	1.14	4.1E-06	1.16	5.0E-10
rs1412829	CDKN2A/2B	9	22043926	1	0.11	T	C	0.72	2.8E-06	0.85	4.2E-04	0.80	2.8E-08
rs4977756	CDKN2A/2B	9	22068652	1	0.22	A	G	0.8	9.7E-06	0.90	2.7E-03	0.87	7.0E-07
rs1063192	CDKN2A/2B	9	22003367	1	0.17	T	C	0.77	2.4E-06	0.90	6.0E-03	0.86	8.4E-07
rs2853668	CLPTM1L/TERT	5	1300025	2	0.31	C	A	1.15	1.7E-03	1.11	5.2E-04	1.12	3.6E-06
rs3731239	C9orf53,CDKN2A	9	21974218	1	0.13	T	C	0.77	6.3E-05	0.87	1.6E-03	0.84	1.3E-06
rs1572072	TNFRSF19	13	24127210	2	0.26	G	T	0.89	1.3E-02	0.92	1.1E-02	0.91	4.8E-04
rs3109384	LOC646388	11	40118598	1	0.26	C	T	0.83	3.3E-05	0.93	1.8E-02	0.89	1.6E-05
rs9928448	ALDOA,PPP4C	16	30072530	2	0.41	T	C	1.17	1.5E-04	1.07	2.4E-02	1.10	6.5E-05
rs10120688	RP11-145E5.4	9	22056499	2	0.28	A	G	0.84	1.8E-04	0.94	4.3E-02	0.91	1.5E-04
rs2877822	MUC13	3	124645034	2	0.04	C	T	0.68	1.1E-04	1.14	5.4E-02	0.96	5.2E-01
rs6671127	LOC100133029,GPR177	1	68571220	1	0.37	A	C	1.2	1.6E-05	1.05	8.5E-02	1.10	1.2E-04
rs10796139	FRMD4A	10	13892298	1	0.36	A	G	0.82	1.3E-05	0.96	1.2E-01	0.91	2.1E-04
rs1331627	NTNG2	9	135091879	1	0.42	C	T	0.84	4.7E-05	1.04	1.3E-01	0.98	2.9E-01
rs11672613	C3	19	6705246	1	0.42	T	C	0.83	1.1E-05	0.96	1.5E-01	0.92	2.4E-04
rs6468749	YWHAZ	8	102008828	1	0.37	T	C	1.21	1.0E-05	1.04	1.5E-01	1.09	2.2E-04
rs12577139	BARX2	11	129301284	2	0.15	C	T	0.84	2.1E-03	1.06	1.5E-01	0.98	5.7E-01
rs7119879	BARX2	11	129305687	2	0.16	G	A	0.84	1.6E-03	1.05	1.9E-01	0.98	4.8E-01
rs1991007		5	55968018	1	0.08	C	A	1.38	2.5E-05	1.05	3.2E-01	1.15	1.3E-03
rs12570170	HK1	10	70801833	1	0.37	G	A	1.19	5.3E-05	1.03	3.5E-01	1.08	2.1E-03
rs2886189	ZBTB16	11	113501655	1	0.30	T	C	0.83	4.3E-05	0.97	3.8E-01	0.93	2.4E-03
rs9820110		3	70469958	1	0.29	G	T	1.24	2.6E-06	1.03	3.8E-01	1.09	7.1E-04
rs17801001	EPHA3	3	89414555	1	0.12	A	C	1.32	7.7E-06	1.03	4.4E-01	1.11	1.7E-03
rs11209216	LOC100133029,GPR177	1	68571431	1	0.44	C	T	1.18	5.5E-05	1.02	4.9E-01	1.07	4.6E-03
rs6795074	EPHA3	3	89516652	1	0.10	T	C	1.38	3.3E-06	1.03	5.1E-01	1.13	1.7E-03
rs9538032		13	58985847	1	0.25	T	C	1.21	5.5E-05	0.98	5.1E-01	1.05	8.2E-02
rs3181088	VCAM1	1	101198708	2	0.11	C	T	1.28	2.6E-04	1.03	5.8E-01	1.10	1.2E-02
rs6800118	MIRN138-1,hsa-mir-138-1	3	44141157	2	0.28	A	G	0.84	1.2E-04	1.02	6.2E-01	0.96	8.0E-02
rs7702277		5	14020756	1	0.12	G	T	1.39	8.0E-08	0.98	6.6E-01	1.10	6.5E-03
rs1296284		5	55934938	1	0.33	G	A	1.21	2.9E-05	1.01	7.0E-01	1.07	7.9E-03
rs2802402	ITM2B	13	47685360	1	0.16	C	T	0.77	2.9E-06	0.99	8.1E-01	0.91	4.4E-03
rs695207	MIR3134,ROD1	9	114056169	1	0.27	T	G	1.2	7.3E-05	0.99	8.2E-01	1.06	3.5E-02
rs189897	ITGA9	3	37518545	2	0.04	A	T	N/A	N/A	1.02	8.3E-01	1.02	8.3E-01
rs2158250	ITGB8	7	20425446	2	0.41	A	G	0.86	4.8E-04	1.00	8.7E-01	0.95	3.7E-02
rs1286041		6	6839192	1	0.17	A	G	1.26	3.1E-05	1.01	8.9E-01	1.08	1.4E-02
rs4714505	LOC100130606,TFEB	6	41648147	1	0.11	C	T	0.71	1.7E-07	1.00	9.3E-01	0.90	4.3E-03
rs7014115	ASPH	8	62649567	1	0.12	T	G	1.33	6.1E-06	1.00	9.5E-01	1.09	1.3E-02
rs4936612		11	121203120	1	0.40	A	G	0.85	6.3E-05	1.00	9.5E-01	0.95	2.7E-02
rs11637457	AGBL1	15	87572506	1	0.16	C	T	0.8	7.7E-05	1.00	9.5E-01	0.93	3.1E-02
rs1324103[d]		6	93901016	1	0.42	A	G	0.84	1.4E-05	1.00	9.6E-01	0.94	1.2E-02
rs9924017	HS3ST4	16	25849321	1	0.36	A	G	1.19	4.7E-05	1.00	9.7E-01	1.06	2.2E-02

[a]Based on hg19.

[b]1 = Selected based on GWAS meta-analysis results; 2 = Selected as an additional candidate based on a-priori literature.

[c]Based on frequency observed among controls in the replication study.

[d]Replaced rs6931820 w/ *P* = 3.47E-06 in GWAS meta.

we conducted a meta-analysis using the fixed effect model to integrate estimates from all studies.

Results

The initial meta-analysis across GWAS included a total of 2,152 cases and 3,740 controls. Results from the meta-analysis are summarized in Supplementary Table S2. As described in the Materials and Methods section, we identified 43 SNPs for replication based on the GWAS meta-analysis. Replication was performed on a total of 4,716 cases and 5,379 controls across four studies (Table 1). Results from this replication effort are summarized in Table 2. In this analysis, the strongest evidence in support of an association with NPC was observed for rs31489 (OR = 0.79; $P = 4.3 \times 10^{-11}$), an intronic SNP within *CLPTM1L* in the *CLPTM1L/TERT* region (chr.5p15.33). This represents a locus not reported in previously published NPC GWAS. Findings for this SNP were consistent in the mainland Chinese and two Taiwanese replication studies and absent from the Malaysian replication study, the smallest of the replication efforts (Supplementary Fig. S1). A second SNP within the *CLPTM1L/TERT* locus (rs2853668; $r^2 = 0.108$ and $D' = 0.917$ with rs 31489 among controls in our replication studies) was also associated with NPC in the replication phase (OR = 1.11; $P = 5.2 \times 10^{-4}$), but the association was no longer statistically significant in analyses that conditioned on rs31489 (OR = 1.05; $P = 0.15$).

In analyses that combined the GWAS and replication studies, findings for rs31489 were strengthened (OR across GWA+replication studies = 0.81; $P_{value} = 6.3 \times 10^{-13}$; Table 2). Some evidence for heterogeneity across studies was observed ($P_{heterogeneity} = 0.035$). Additional associations ($P \leq 1 \times 10^{-7}$) were observed in our combined GWA plus replication studies meta-analysis for rs6774494 ($P = 1.5 \times 10^{-12}$; *MECOM* gene region), rs9510787 ($P = 5.0 \times 10^{-10}$; *TNFRSF19* gene region), rs1412829, rs4977756, and rs1063192 ($P = 2.8 \times 10^{-8}$, $P = 7.0 \times 10^{-7}$, and $P = 8.4 \times 10^{-7}$, respectively; *CDKN2A/2B* gene region; Table 2 and Supplementary Fig. S1).

Discussion

We report herein results from a meta-analysis of NPC GWAS followed by replication studies across three regions in Asia. A novel association was observed within the *CLPTM1L/TERT* locus. This finding is of note given that SNPs in this region were reported from GWAS conducted for numerous other cancers, including lung, bladder, pancreas, testis, and central nervous system (10). A recent meta-analysis of 85 studies including over 490,000 subjects that evaluated 67 *TERT/CLPTM1L* locus polymorphisms and 24 tumor types identified 11 SNPs with strong cumulative evidence for an association with at least one cancer type. rs31489 was one of these SNPs and was found to have strong cumulative evidence for association with testicular cancer among Caucasians and moderate cumulative evidence for association with Asian lung cancer (10). Furthermore, a review of the literature identified candidate gene studies (two that evaluated SNPs and a third that evaluated a microsatellite marker) that reported an association between polymorphisms within the *CLPTM1L/TERT* locus and NPC (11–13). Two of the three SNPs evaluated in these studies are in LD with rs31489 (rs401681 $r^2 = 0.427$ in 1 kG ASN and 0.512 in 1 kG CHB; rs402710 $r^2 = 0.433$ in 1 kG ASN and 0.569 in 1 kG CHB). The third SNP is not in LD with rs31489, suggesting the possibility for the existence of greater than one independent susceptibility variant within the *CLPTM1L/TERT* locus (rs2736098 $r^2 = 0.016$ in 1 kG ASN and 0.049 in 1 kG CHB). Our findings in the *CLPTM1L/TERT* locus gain added significance given the role of TERT in telomere length regulation (14), the finding that telomerase overexpression is observed in NPC (15), and that the EBV protein LMP1, a protein frequently expressed in NPC, activates *TERT* expression and enhances telomerase activity (16, 17). We did observe evidence for possible heterogeneity in effect observed for rs31489 across study populations ($P_{heterogeneity} = 0.035$). The evidence for heterogeneity was of marginal statistical significance and was driven primarily by the two Malaysian studies included in our effort. It is unclear at this time whether our findings reflect true heterogeneity, differential misclassification of ethnicity in the Malaysian studies, or a chance finding. This observation deserves further consideration in future studies.

Additional associations ($P \leq 1 \times 10^{-7}$) were observed in our combined GWA plus replication studies meta-analysis for rs6774494 ($P = 1.5 \times 10^{-12}$; *MECOM* gene region), rs9510787 ($P = 5.0 \times 10^{-10}$; *TNFRSF19* gene region), rs1412829, rs4977756, and rs1063192 ($P = 2.8 \times 10^{-8}$, $P = 7.0 \times 10^{-7}$, and $P = 8.4 \times 10^{-7}$, respectively; *CDKN2A/2B* gene region; Table 2 and Supplementary Fig. S1). These associations were previously reported from the Mainland China NPC GWAS and their potential biological implications discussed (6); our data provide support for these associations.

Strengths of our study include the fact that it evaluated associations with NPC across multiple GWAS and the large size of its replication effort. Limitations include the inability to further investigate potential heterogeneity of effects by exposure status or geographic/ethnic groups. Future studies should explore the associations reported herein in additional populations with differing ethnic makeup.

In conclusion, our GWAS meta-analysis and replication effort has identified an additional susceptibility locus for NPC within the *CLPTM1L/TERT* region of chromosome 5p15.33 and provides support for several previously reported NPC susceptibility loci.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acknowledgments

The authors thank the full membership of the International NPC Genetics Working Group who contributed to this publication. They include Jin-Xin Bei; Kai-Ping Chang (Department of Otolaryngology, Chang Gung Memorial Hospital at Lin-Kou, Taoyuan, Taiwan); Yu-Sun Chang; Chien-Jen Chen; Yoon-Ming Chin; Charles Chung (Center for Genomics Research, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD); Allan Hildesheim; Wan-Lun Hsu; Alan Soo-Beng Khoo; Jian-Jun Liu; Pei-Jen Lou; James D. McKay; Ching-Ching Ng; Kin-Choo Pua; Lee-Chu See (Department of Biostatistics and Chang Gung Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan); Wen-Hui Su; Soo-Hwang Teo; Ngan-Ming Tsang (Department of Radiation Oncology, Chang Gung Memorial Hospital at Lin-Kou, Taoyuan, Taiwan); Ka-Po Tse (Chang Gung Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan); Meredith Yeager (Center for Genomics Research, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD); Chia-Jung Yu (Chang Gung Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan); Kai Yu; Yi-Xin Zeng.

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Grant Support

This work was supported by the National High Technology Research and Development Program of China (863; 2012AA02A501, 2012AA02A206; J.-X. Bei and Y. Zeng), the Major State Basic Research Development Program of China (973; 2011CB504302; to J.-X. Bei and Y. Zeng), the National Natural Science Foundation of China (81222035, 81101544; to J.-X. Bei and Y. Zeng), the Pearl River Nova Program and the Agency for Science, Technology, and Research of Singapore (to J.-X. Bei and J. Liu), the Ministry of Education of Taiwan, the Malaysian Ministry of Higher Education-High Impact Research (H-50001-A000023; to C. Ng and Y. Chin), and by the Intramural Research Program of the U.S. National Cancer Institute (to A. Hildesheim and K. Yu).

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Received March 17, 2015; revised September 29, 2015; accepted October 2, 2015; published OnlineFirst November 6, 2015.

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