Journal of Human Genetics (2016) 61, 793–796

www.nature.com/jhg

ORIGINAL ARTICLE

A significant association between rs8067378 at 17q12 and invasive cervical cancer originally identified by a genome-wide association study in Han Chinese is replicated in a Japanese population

Kiyonori Miura[1], Hiroyuki Mishima[2], Michio Yasunami[3], Masanori Kaneuchi[1], Michio Kitajima[1], Shuhei Abe[1], Ai Higashijima[1], Naoki Fuchi[1], Shoko Miura[1], Koh-Ichiro Yoshiura[2] and Hideaki Masuzaki[1]

In this study, associations between invasive cervical cancer and four cervical cancer susceptibility loci (rs13117307 at 4q12, rs8067378 at 17q12, and rs4282438 and rs9277952 at 6p21.32) in the Han Chinese population were investigated in a Japanese population. Human leukocyte antigen (*HLA)-DPB1* alleles were also investigated for their association with cervical cancer risk in the Japanese population. After receiving written informed consent, 214 unrelated Japanese women with invasive cervical cancer and 288 cancer-free Japanese women were recruited, and DNA samples were obtained (study protocol approved by Institutional Review Board of Nagasaki University). Of the four single-nucleotide polymorphisms, rs8067378 showed a significant association with invasive cervical cancer ($P = 0.0071$). Under a recessive model, the minor allele G of rs8067378 contributed to the risk of invasive cervical cancer (odds ratio = 2.92, 95% confidence interval = 1.40–6.36; $P = 0.0021$). No association was detected between *HLA-DPB1* alleles and cervical cancer risk in the Japanese population. In conclusion, we show for the first time, to the best of our knowledge, that an association between increased risk of invasive cervical cancer and rs8067378 in the Han Chinese population is replicated in a Japanese population. In addition, Japanese women with the GG genotype of rs8067378 are a candidate high-risk group for invasive cervical carcinoma.
Journal of Human Genetics (2016) **61,** 793–796; doi:10.1038/jhg.2016.50; published online 19 May 2016

INTRODUCTION

Cervical cancer is the third most common cancer in women worldwide,[1] and is caused by persistent infection with 1 of approximately 15 carcinogenic human papillomaviruses (HPV). Although oncogenic HPV infection is recognized as a major risk factor of cervical cancer, oncogenic HPV infection alone is insufficient to develop cervical cancer. HPV infections are very common in women having an experience of sexual intercourse, though most of them disappear naturally within several years. Therefore, cervical cancer develops in only a small proportion of women with HPV infections.[2] These data indicate that pathogenesis of cervical cancer depends on the interaction between carcinogenic factor of HPV itself and host factors related with persistent infection of HPV and/or cervical cancer development. As genetic factor of host, the existence of susceptibility loci for cervical cancer is suggested.[3] Therefore, genetic analysis is promising approach to clarify the pathogenesis of cervical cancer and will provide useful information for both disease prevention and therapeutic management of invasive cervical cancer.[4]

To date, many case–control studies have reported the association of host genetic variations with cervical cancer, for example, single-nucleotide polymorphisms (SNPs) in human leukocyte antigens (*HLA*) class II, Fanconi anemia complementation group A (*FANCA*), interferon regulatory factor 3 (*IRF3*), sulfatase 1 (*SULF1*), deoxyuridine triphosphate (*DUT*), general transcription factor IIH, polypeptide 3 (*GTF2H4*), interferon gamma (*IFNG*), epidermal dysplasia verruciformis (EV)-associated *EVER1/EVER2*, peroxiredoxin 3 (*PRDX3*) and ribosomal protein S19 (*RPS19*).[4–8] Recently, three genome-wide association studies (GWAS) have identified susceptibility loci for cervical cancer.[9–11] Our previous GWAS in the Japanese population did not identify susceptibility loci for cervical cancer.[11] However, two other GWAS in different populations successfully identified cervical cancer susceptibility loci. One GWAS in the Han Chinese population identified three cervical cancer susceptibility loci: 4q12 (rs13117307), 17q12 (rs8067378) and 6p21.32 (rs4282438 and rs9277952),[10] while the another study in a US population identified three cervical cancer susceptibility loci at 6p21.32 (rs2516448,

[1]Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [2]Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan and [3]Department of Pediatric Infectious Disease, Institute of Tropical Medicine, Nagasaki, Japan
Correspondence: Dr K Miura, Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
E-mail: kiyonori@nagasaki-u.ac.jp
Received 17 December 2015; revised 12 April 2016; accepted 13 April 2016; published online 19 May 2016

rs9272143 and rs3117027).[9] However, the results of the above three different GWAS did not overlap. Cervical cancer susceptibility loci in each ethnicity varied; and therefore, it is necessary to confirm whether susceptibility loci in one population are specifically replicated in another.[4]

In this study, we investigated whether four cervical cancer susceptibility loci (rs13117307 at 4q12, rs8067378 at 17q12, and rs4282438 and rs9277952 at 6p21.32) identified in the Han Chinese population were associated with cervical cancer in a Japanese population. We also investigated whether *HLA-DPB1* alleles are associated with cervical cancer risk in the Japanese population, because this association was found to be significant in Chinese and Taiwanese populations.[12–14]

MATERIALS AND METHODS

Subjects and samples

The study participants, who were overlapped with our previous GWAS,[11] were 214 unrelated Japanese women with uterine cervical cancer (mean age, 52 years; age range, 29–70 years). By experienced pathologists at Nagasaki University Hospital, all tumor samples were diagnosed as invasive squamous cell carcinoma, before or after surgery.[11] Overall, 288 cancer-free women, who had no abnormal cytology of the uterine cervix, were used as the control population. There was no significant difference in age between 214 cases of invasive cervical cancer and 288 cancer-free control women. All samples were obtained after receiving written informed consent, and the study protocol was approved by the Institutional Review Board for Ethical, Legal and Social Issues of Nagasaki University.

Out of 214 Japanese women with uterine cervical cancer, cervical specimens were obtained from 156 cases before hysterectomy, and HPV genotyping was performed. We considered 16 HPV genotypes (16, 18, 31, 33, 35, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) as high risk related to uterine cervical cancer. Of the 156 cases, HPV 16 was detected in 73 (46.8%), HPV 18 in 19 (12.2%), HPV 52 in 21 (13.5%) and the other HPV high-risk genotypes in 43 (27.5%). In the remaining 58 cases, HPV genotypes were unknown because the women had already undergone hysterectomies before study inclusion.

SNP genotyping

SNPs of rs13117307 (position; chr4: 56,446,497), rs8067378 (position; chr17: 35,304,874), rs4282438 (position; chr6: 33,180,150) and rs9277952 (position; chr6: 33,312,252) were genotyped by direct sequencing analysis of PCR products. Forward and reverse PCR primer sequences for each SNP are as follows: CCTCCTTGAGAAATTCTTAAA and TTCTTCTCCTGTAGCTGATATC for rs13117307, CCTTCCTGCCTTCTTCCTCCTTT and GATGACTGGTGAAATAAGCAGC for rs8067378, AGAAAGAGGACTTTGAGTCTTTC and GGCTGCTCTGGGGCTTGGATA for rs4282438 and CAGCCTGAGCAACAAAGCCAG and ACAGCAACCCTGTGAGGTGGG for rs9277952. Primer sequences for each SNP were as follows: AACTAACATAATACAGGTT for rs13117307, AACTGACAGATTAGTGTGA for rs8067378, AGGTATATGCCCTTATGCA for rs4282438 and CCATTTTACAGAGAGGAAA for rs9277952. PCR was performed using the Gene Amp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) with the conditions of 2 min at 95 °C, and 35 cycles of 10 s at 95 °C, 20 s at 56 °C, 30 s at 72 °C and 2 min at 72 °C. PCR products were separated by 3100 Genetic Analyzer (Applied Biosystems).

HLA-DPB1 genotyping

HLA-DPB1 allele genotyping was determined by sequence-based typing, including the PCR amplification of genomic DNA and direct sequencing. Amplification was performed using KAPA HiFi HotStart DNA polymerase (Kapa Biosystems, Wilmington, MA, USA) and specific amplification primers for *HLA-DPB1* exon 2 were as follows:

*HLA-DPB1*exon2F02: 5′-CCACAGAACTCGGTACTAGGAA-3′,
*HLA-DPB1*exon2R02: 5′-AGGCCAACCCGGCTGCTCCTG-3′.

Direct sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with specific internal primers 5′-CCTATTTTAAAATCCAGCCCTGG-3′ and/or 5′-GGCTGCTCCTGCGCCCTGG-3′. Electropherograms were analyzed using the Assign-ATF software (Conexio Genomics, Fremantle, Australia).

Statistical analysis

Four SNPs were analyzed by the Haploview4.1 software for linkage disequilibrium (Broad institute of MIT, Harvard, MA, USA), with particular focus on rs4282438 and rs9277952 at 6p21.31.[15]

Fisher's exact tests of allele counts in four loci, and the Fisher's exact tests of genotypes of rs8067378 under dominant and recessive models were performed using R 3.1.2 (http://www.R-project.org/).[16] A *P*-value of less than 0.0125 indicated significance using Bonferroni correction.

Fisher's exact test was performed for *HLA-DPB1*. There were 12 alleles with allele frequency >1% in control or cervical cancer group or both, and those were used for allelic association one by one. A *P*-value of less than 0.00416 indicates significance with Bonferroni correction.

RESULTS

Association between each SNP and invasive cervical cancer

Table 1 summarizes the association between each SNP and invasive cervical cancer. In addition, the SNP tendencies in the Japanese population were listed in Table 1. Of the four SNPs investigated (rs13117307, rs8067378, rs4282438 or rs9277952), one locus (rs8067378 at 17q12) showed a significant association ($P = 0.00715$, odds ratio (OR) = 1.47, 95% confidence interval (CI): 1.12–1.94). In contrast, there was no association between the other SNPs (rs13117307, rs4282438 or rs9277952) and invasive cervical cancer (*P*-value >0.0125 and OR >1.0, respectively). Because only weak linkage disequilibrium was observed between rs4282438 and rs9277952 at 6p21.31, with $D' = 0.59$ and $r^2 = 0.26$, a *P*-value of less than 0.0125 (0.05/4) is strict value as significance, when a Bonferroni correction was applied.

rs8067378 is significantly associated with increased risk of invasive cervical cancer

Table 2 summarizes the estimates of the main effects of rs8067378. Under the recessive model, the minor allele G of rs8067378 was significantly associated with increased risk of invasive cervical cancer (OR = 2.92, 95% CI = 1.40–6.36; *P*-value = 0.0021). In contrast, under the dominant model, there was no significant association between the minor allele G of rs8067378 and increased risk of invasive cervical cancer (OR = 1.39, 95% CI = 0.96–2.02; *P*-value = 0.0715).

Table 1 Fisher's exact test of allele counts

Loci	*Major allele*	*Allele counts*[a]	*Minor allele*	*Allele counts*[a]	P-*value*	*Odds ratio*	*95% CI*
rs13117307	C	358:487	T	70:89	0.7269	1.07	0.76–1.51
rs8067378	A	287:432	G	141:144	0.0071	1.47	1.12–1.94
rs4282438	T	259:353	G	169:221	0.7934	1.04	0.81–1.35
rs9277952	G	220:312	A	208:264	0.4061	1.12	0.87–1.44

Abbreviation: CI, confidence interval.
[a]Invasive cervical carcinoma: control.

Table 2 Effects of rs8067378 under dominant and recessive models

	Genotypes		*Dominant model (AA vs AG/GG)*			*Recessive model (AA/AG vs GG)*		
Group	*AA/AG/GG*[a]	*HWE P-value*[b]	*Odds ratio*	*95% CI*	*P-value*[c]	*Odds ratio*	*95% CI*	*P-value*[c]
Invasive cervical cancer	99/89/26	0.39	1.39	0.96–2.02	0.0715	2.92	1.41–6.36	0.0021
Control	157/118/13	0.11						

Abbreviations: CI, confidence interval; HWE, Hardy–Weinberg equilibrium.
[a]Case number of each genotype.
[b]Hardy Weinberg Equilibrium test.
[c]Fisher's exact test.

Table 3 Association between *HLA-DPB1* alleles and the risk of invasive cervical cancer

			Allele frequency			
HLA-DPB1 alleles	*Odds ratio*	*95% CI*	*Invasive cervical cancer group*	*Control group*	*Both group*	P-*value*[a]
DPB1*01:01	0.45	na		0.002	0.001	Excluded[b]
DPB1*02:01	1.28	0.95–1.72	0.250	0.207	0.225	0.109
DPB1*02:02	0.91	0.47–1.78	0.035	0.038	0.037	0.866
DPB1*03:01	2.22	1.15–4.29	0.056	0.026	0.039	0.0199
DPB1*04:01	0.76	0.43–1.36	0.044	0.057	0.052	0.391
DPB1*04:02	0.74	0.45–1.21	0.063	0.083	0.075	0.275
DPB1*05:01	0.96	0.75–1.25	0.379	0.387	0.383	0.793
DPB1*06:01	0.77	0.22–2.64	0.009	0.012	0.011	0.767
DPB1*09:01	0.89	0.60–1.32	0.110	0.122	0.117	0.619
DPB1*13:01	0.98	0.39–2.45	0.019	0.019	0.019	1.000
DPB1*14:01	0.53	0.17–1.71	0.009	0.017	0.014	0.416
DPB1*16:01	0.09[c]	0.01–1.56[c]	0.000	0.012	0.007	0.0226
DPB1*17:01	0.27	na	0.000	0.003	0.002	Excluded
DPB1*19:01	1.35	0.39–4.69	0.012	0.009	0.010	0.751
DPB1*21:01	0.45	na	0.000	0.002	0.001	Excluded
DPB1*38:01	9.48	na	0.007	0.000	0.003	Excluded
DPB1*41:01	4.06	na	0.007	0.002	0.004	Excluded
DPB1*108:01	0.45	na	0.000	0.002	0.001	Excluded

Abbreviations: CI, confidence interval; na, not applicable.
[a]Fisher's exact test.
[b]Data were excluded from statistical test because of low frequency.
[c]Haldane's modification of Woolf's formula was applied. 95% CI for odds ratio was calculated using chi-square statistic.

Association between HLA-DPB1 alleles and invasive cervical cancer
Table 3 summarizes the finding that no association was observed between *HLA-DPB1* alleles and risk of invasive cervical cancer in the Japanese population.

DISCUSSION

This study confirmed an association between invasive cervical cancer and rs8067378 identified at 17q12 in the Han Chinese population in a Japanese population. However, no association was detected between *HLA-DPB1* alleles and the risk of cervical cancer in the Japanese population, though a significant association between *HLA-DPB1* alleles and cervical cancer risk had previously been observed in Chinese and Taiwanese populations.

Cervical cancer susceptibility locus, rs8067378, in both Japanese and Han Chinese populations is located 9.5 kb downstream of gasdermin B (*GSDMB*) and encodes the cancer-associated gasdermin-like protein (*GSDML*).[10] The expression of *GSDMB* is detected in human cancer tissues, for example, gastric, hepatic and cervical cancers. *GSDML* seems to be related to the cervical cancer-cell proliferation and the cervical cancer development.[4,10,17] Indeed, replication of a susceptibility locus in the *GSDMB* region in two different populations suggests an essential role for tumor-cell proliferation, supporting the hypothesis that an inherited carcinogenic factor in host has an important part in determining the risk for cervical cancer, probably by influencing the mechanism responsible for persistent infection and the integration of HPV.[4,10,17]

Subsequently, we estimated the main effects of rs8067378 in the pathogenesis of invasive cervical cancer. Under the recessive model, the minor allele G of rs8067378 was significantly associated with increased risk of invasive cervical cancer (OR = 2.92, 95% CI = 1.41–6.36; *P*-value = 0.0021), suggesting that Japanese women with GG genotype of rs8067378 at 17q12 are a candidate high-risk group of invasive cervical carcinoma. Therefore, identification of the genes, which take part in persistent infection of HPV and cervical cancer development, bring important information for both disease prevention and therapeutic management of invasive cervical cancer.[4]

Out of four SNPs (rs8067378 at 17q12, rs13117307 at 4q12, and rs8067378 and rs4282438 at 6p21.32) previously associated with cervical cancer risk in the Han Chinese population,[10] only rs8067378 was also found to be associated with susceptibility to cervical cancer in the Japanese population; the associations between rs13117307, rs4282438 and rs9277952 and cervical cancer were not replicated. However, as the ORs of the four SNPs were generally low in Han Chinese (1.1–1.3),[10] we examined the SNP tendencies in the Japanese

population. ORs of the four minor alleles were 1.47 for rs8067378, 1.07 for rs13117307, 1.04 for rs4282438 and 1.12 for rs9277952 (Table 1). This compares with 1.18, 1.26, 0.75 and 0.85, respectively, in Han Chinese.[10] Therefore, the minor alleles of rs4282438 and rs9277952 at 6p21.32 showed different directions of effect between the present study and the Han Chinese GWAS, while the minor alleles of rs8067378 at 17q12 and rs13117307 at 4q12 showed the same direction. Thus, the minor allele of rs8067378 is associated with susceptibility to cervical cancer in both Japanese and Han Chinese populations. Although the association between rs13117307 and cervical cancer did not reach significance in the present study, this is conceivably because of the low sample size.

Many disease-associated genetic variants in one population are either inconsistent or failed to be independently replicated in other populations.[4] Shi *et al.*[10] genotyped 1374 cases of invasive cervical carcinoma and 3135 controls using the Affymetrix Axiom Genome-Wide CHB1 Array (Affymetrix, Santa Clara, CA, USA), and four SNPs (rs13117307, rs8067378, rs4282438 and rs9277952) were identified as cervical cancer susceptibility loci in a Han Chinese population. However, of these four cervical cancer susceptibility loci, the Affymetrix Genome-Wide SNP Array 6.0 (Affymetrix) we used in our GWAS did not include three SNPs (rs13117307, rs8067378 and rs4282438). To date, three GWAS regarding cervical cancer have been reported,[9–11] but there was no consistent association of SNPs across the different GWAS.[4] Because different SNP microarrays were used in these GWAS, coverage of genetic variants was non-identical between the studies. This is a limitation of high-throughput SNP microarray analysis. As a GWAS using SNP array provides a part of susceptibility loci for cervical cancer, it may be insufficient to clarify the whole pathogenesis of cervical cancer.[4,18] Therefore, to resolve the limitations of SNP microarray analysis and to identify all cervical cancer susceptibility loci, SNP imputation or more advanced high-throughput analysis (such as exome analysis using a next-generation sequencing approach) will be necessary.

In conclusion, we report for the first time an association between invasive cervical cancer and rs8067378 at 17q12 first identified in the Han Chinese population, in a Japanese population. Furthermore, Japanese women with GG genotype of rs8067378 could be a candidate high-risk group of invasive cervical carcinoma. The studies on genes participating in persistent HPV infection and/or cervical cancer development should be continued. Further information regarding cervical cancer susceptibility loci will increase our understanding of pathogenesis of cervical cancer and may provide new screening strategies to prevent invasive cervical cancer.[4]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Yasuko Noguchi for her technical assistance in sample preparation. KM, SA, AH, NF, SM and HM were supported by JSPS KAKENHI, grant numbers 26462495, 16K20199, 26861333, 16K20198, 15K10677 and 25462563, respectively.

Author contributions: KM wrote the main manuscript text and performed genotyping analysis. HiroM performed statistical analysis, and interpreted the data. MaK, MiK, SA, AH and FN were attending doctors for the cases of cervical cancer, and prepared DNA samples for genotyping analysis. SM performed genotyping analysis, and interpreted the data. K-iY and HideM interpreted the data, performed critical revision of the manuscript and supervised experiments. All authors reviewed the manuscript.

1 Arbyn, M., Castellsagué, X., de Sanjosé, S., Bruni, L., Saraiya, M., Bray, F. *et al.* Worldwide burden of cervical cancer in 2008. *Ann. Oncol.* **22**, 2675–2686 (2011).
2 Muñoz, N., Bosch, F. X., de Sanjosé, S., Herrero, R., Castellsagué, X., Shah, K. V. *et al.* International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N. Engl. J. Med.* **348**, 518–527 (2003).
3 Magnusson, P. K., Lichtenstein, P. & Gyllensten, U. B. Heritability of cervical tumours. *Int. J. Cancer* **88**, 698–701 (2000).
4 Chen, D. & Gyllensten, U. Lessons and implications from association studies and post-GWAS analyses of cervical cancer. *Trends Genet.* **31**, 41–54 (2015).
5 Matsumoto, K., Maeda, H., Oki, A., Takatsuka, N., Yasugi, T., Furuta, R. *et al.* for Japan HPV And Cervical Cancer (JHACC) Study Group. HLA class II DRB1*1302 allele protects against progression to cervical intraepithelial neoplasia grade 3: a multicenter prospective cohort study. *Int. J. Gynecol. Cancer* **22**, 471–478 (2012).
6 de Freitas, A. C., Gurgel, A. P., Chagas, B. S., Coimbra, E. C. & do Amaral, C. M. Susceptibility to cervical cancer: an overview. *Gynecol. Oncol.* **126**, 304–311 (2012).
7 Zhang, X., Zhang, L., Tian, C., Yang, L. & Wang, Z. Genetic variants and risk of cervical cancer: epidemiological evidence, meta-analysis and research review. *BJOG* **121**, 664–674 (2014).
8 Chen, D. & Gyllensten, U. Systematic investigation of contribution of genetic variation in the HLA-DP region to cervical cancer susceptibility. *Carcinogenesis* **35**, 1765–1769 (2014).
9 Chen, D., Juko-Pecirep, I., Hammer, J., Ivansson, E., Enroth, S., Gustavsson, I. *et al.* Genome-wide association study of susceptibility loci for cervical cancer. *J. Natl Cancer Inst.* **105**, 624–633 (2013).
10 Shi, Y., Li, L., Hu, Z., Li, S., Wang, S., Liu, J. *et al.* A genome-wide association study identifies two new cervical cancer susceptibility loci at 4q12 and 17q12. *Nat. Genet.* **45**, 918–922 (2013).
11 Miura, K., Mishima, H., Kinoshita, A., Hayashida, C., Abe, S., Tokunaga, K. *et al.* Genome-wide association study of HPV-associated cervical cancer in Japanese women. *J. Med. Virol.* **86**, 1153–1158 (2014).
12 Wu, Y., Liu, B., Lin, W., Xu, Y., Li, L., Zhang, Y. *et al.* Human leukocyte antigen class II alleles and risk of cervical cancer in China. *Hum. Immunol.* **68**, 192–200 (2007).
13 Liang, J., Xu, A., Xie, Y., Awonuga, A. O. & Lin, Z. Some but not all of HLA-II alleles are associated with cervical cancer in Chinese women. *Cancer Genet. Cytogenet.* **187**, 95–100 (2008).
14 Yang, Y. C., Chang, T. Y., Chen, T. C., Lin, W. S., Chang, S. C. & Lee, Y. J. Genetic susceptibility to cervical squamous cell carcinoma is associated with HLA-DPB1 polymorphisms in Taiwanese women. *Cancer Immunol. Immunother.* **64**, 1151–1157 (2015).
15 Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265 (2005).
16 R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2014 (http://www.R-project.org/).
17 Sun, Q., Yang, J., Xing, G., Sun, Q., Zhang, L. & He, F. Expression of GSDML associates with tumor progression in uterine cervix cancer. *Transl. Oncol.* **1**, 73–83 (2008).
18 Barabási, A. L. Network medicine–from obesity to the 'diseasome'. *N. Engl. J. Med.* **357**, 404–407 (2007).