The Journal of Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America hivma hiv medicine association OXFORD

HLA and *KIR* Associations of Cervical Neoplasia

Xiao Bao,[1,2,3] Aimee L. Hanson,[4,5,6] Margaret M. Madeleine,[13] Sophia S. Wang,[14] Stephen M. Schwartz,[13] Felicity Newell,[3] Ulrika Pettersson-Kymmer,[18,19] Kari Hemminki,[20,23] Sven Tiews,[25] Winfried Steinberg,[25] Janet S. Rader,[15] Felipe Castro,[23,24] Mahboobeh Safaeian,[16] Eduardo L. Franco,[26] François Coutlée,[27] Claes Ohlsson,[21,22] Adrian Cortes,[4] Mhairi Marshall,[3] Pamela Mukhopadhyay,[4] Katie Cremin,[4] Lisa G. Johnson,[13] Suzanne M. Garland,[7,8,9] Sepehr N. Tabrizi,[7,8,9] Nicolas Wentzensen,[16] Freddy Sitas,[10,11,12] Cornelia Trimble,[17] Julian Little,[28] Maggie Cruickshank,[29] Ian H. Frazer,[5,6] Allan Hildesheim,[16] Matthew A. Brown,[3,a] Emma L. Duncan,[3,a] YingPu Sun,[1,2,a] and Paul J. Leo[3,a]

[1]Center for Reproductive Medicine and [2]Henan Key Laboratory of Reproduction and Genetics, First Affiliated Hospital of Zhengzhou University, China; [3]Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, [4]University of Queensland Diamantina Institute, and [5]Faculty of Medicine and Biomedical Sciences, University of Queensland, and [6]Translational Research Institute, Princess Alexandra Hospital, Woolloongabba, [7]Western Pacific Regional Human Papillomavirus Laboratory Network, Department of Microbiology and Infectious Diseases, and [8]Murdoch Children's Research Institute, Royal Children's Hospital, and [9]Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, [10]Cancer Council NSW, Sydney, [11]Sydney School of Public Health, University of Sydney, Camperdown, and [12]School of Public Health and Community Medicine, University of New South Wales, Kensington, Australia; [13]Program in Epidemiology, Fred Hutchinson Cancer Research Center, Seattle, Washington; [14]Department of Population Sciences, Beckman Research Institute, City of Hope, Duarte, California; [15]Department of Obstetrics and Gynecology, Medical College of Wisconsin, Milwaukee; [16]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, and [17]Center for Cervical Dysplasia, Johns Hopkins University, Baltimore, Maryland; [18]Department of Pharmacology and Clinical Neuroscience and [19]Department of Public Health and Clinical Medicine, Umeå University, Umeå, [20]Center for Primary Health Care Research, Lund University, Lund, and [21]Internal Medicine and Clinical Nutrition and [22]Center for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [23]Division of Molecular Genetic Epidemiology and [24]Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, and [25]MHC Laboratory for Cytopathology, Dr Steinberg, Soest, Germany; [26]Division of Cancer Epidemiology, McGill University, and [27]Département de Microbiologie, Infectiologie et Immunologie, Centre Hospitalier de l'Université de Montréal, Montréal, and [28]School of Epidemiology and Public Health, Faculty of Medicine, University of Ottawa, Ottawa, Canada; and [29]Division of Medical Education, University of Aberdeen, United Kingdom

Background. Cervical cancer is the fourth most common cancer in women, and we recently reported human leukocyte antigen (*HLA*) alleles showing strong associations with cervical neoplasia risk and protection. HLA ligands are recognized by killer immunoglobulin-like receptors (KIRs) expressed on a range of immune cell subsets, governing their proinflammatory activity. We hypothesized that the inheritance of particular *HLA-KIR* combinations would increase cervical neoplasia risk.

Methods. Here, we used *HLA* and *KIR* dosages imputed from single-nucleotide polymorphism genotype data from 2143 cervical neoplasia cases and 13 858 healthy controls of European decent.

Results. The following 4 novel *HLA* alleles were identified in association with cervical neoplasia, owing to their linkage disequilibrium with known cervical neoplasia–associated *HLA-DRB1* alleles: *HLA-DRB3*9901* (odds ratio [OR], 1.24; $P = 2.49 \times 10^{-9}$), *HLA-DRB5*0101* (OR, 1.29; $P = 2.26 \times 10^{-8}$), *HLA-DRB5*9901* (OR, 0.77; $P = 1.90 \times 10^{-9}$), and *HLA-DRB3*0301* (OR, 0.63; $P = 4.06 \times 10^{-5}$). We also found that homozygosity of *HLA-C1* group alleles is a protective factor for human papillomavirus type 16 (HPV16)–related cervical neoplasia (*C1/C1;* OR, 0.79; $P = .005$). This protective association was restricted to carriers of either *KIR2DL2* (OR, 0.67; $P = .00045$) or *KIR2DS2* (OR, 0.69; $P = .0006$).

Conclusions. Our findings suggest that *HLA-C1* group alleles play a role in protecting against HPV16-related cervical neoplasia, mainly through a *KIR*-mediated mechanism.

Keywords. Cervical neoplasia; human leukocyte antigens (HLA); killer immunoglobulin-like receptors (KIRs); HPV16-related cervical neoplasia.

Cervical cancer is the fourth most common cancer in women, with >500 000 new cases presenting worldwide in 2012, and accounts for 7.5% of cancer deaths among female individuals [1]. Its impact is particularly high among young women, ranking as the second commonest cancer affecting women aged 20–39 years [2]. Cervical cancer results from chronic infection with human papillomavirus (HPV), with the HPV genome detected in nearly all cases of cervical cancer. Of the different HPV types, HPV16 and HPV18 are most frequently involved, and together they account for approximately 70% of cervical cancers worldwide [3]. Whereas infection with HPV is essentially universal, most cervical HPV infections are cleared by the immune system [4, 5], and only approximately 1% of women with cervical HPV infection develop cervical cancer [6].

Genetic factors strongly influence the persistence of HPV infection and the risk of cervical cancer. HPV persistence is associated with the monogenic disorders epidermodysplasia verruciformis and WHIM syndrome, from mutations in *EVER1/2* and *CXCR4*, respectively [7, 8]. The only robust common variant genetic associations with cervical cancer are with genes of the major histocompatibility complex (MHC), in particular *HLAs*. We demonstrated that the haplotypes *HLA-DRB1*1501/HLA-DQB1*0602/HLA-DQA1*0102* and *HLA-DQA1*0301/*

Received 15 March 2018; editorial decision 31 July 2018; accepted 6 August 2018; published online August 7, 2018.

[a]M. A. B., E. L. D., Y. S., and P. J. L. contributed equally to this work.

Correspondence: Y. P. Sun, Center for Reproductive Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China (syp2008@vip.sina.com).

The Journal of Infectious Diseases® 2018;218:2006–15

© The Author(s) 2018. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.

DOI: 10.1093/infdis/jiy483

*HLA-DRB1*0401* increase the risk of HPV-associated cervical neoplasia and that the allele *HLA-B*15* and haplotype *HLA-DRB1*1301/HLA-DQB1*0603* are protective. Of note, *HLA-DRB1*1301/HLA-DQA1*0103/HLA-DQB1*0603* is associated with protection from oral and pharyngeal cancer, particularly among HPV-positive cases [9]. We showed that the HLA risks of cervical neoplasia were determined by amino acids at positions 13 and 71 in pocket 4 of HLA-DRB1 and position 156 in HLA-B [10]. Common genetic variant contribution to cervical neoplasia susceptibility is substantial (36%) [10], although a large component of the heritability has yet to be elucidated.

HLA proteins are critical for antigen presentation to effector cells of the adaptive immune system [11]. HLA class I complexes are expressed on all nucleated cells and present endogenous, intracellular-derived antigens, as well as pathogen-derived peptides (as with viral infection), with a residue length of 8–10 amino acids. These are recognized by $CD8^+$ T cells that can engage foreign peptides through their T-cell receptor. Conversely, natural killer (NK) cells, a component of the innate immune system, are able to respond to downregulated surface HLA, a consequence of the immune evasion strategy of some viruses to avoid $CD8^+$ T-cell recognition. Once activated, these lymphocytes can kill the antigen-presenting cell via release of cytotoxic granules. HLA class II complexes typically present extracellular-derived antigens, such as bacterial pathogens. After endocytosis, these proteins are processed and presented on the cell surface bound to MHC class II to initiate an immune response from $CD4^+$ cells.

Interaction between HLA, viral epitopes, and killer immunoglobulin-like receptors (KIR) expressed on NK cells lead either to activation or inhibition of NK cell cytotoxic activity. KIRs are expressed on all NK cells and a minority of T cells (including some $CD4^+$, $CD8^+$, and γδ cells). Seventeen *KIR* genes have been identified, encoded within the leukocyte receptor complex on chromosome 19q13.4, all of which share significant homology (85%–99% DNA sequence similarity) [12, 13]. They are encoded in variable gene content haplotypes with activating and inhibitory counterparts. Different inhibitory and activating KIRs demonstrate specificity for different HLA subgroups, providing fine-tuning of NK and KIR-bearing T-cell responses [14].

Variability at the *KIR* locus includes allelic, gene combination (haplotypic), and expression level differences, with the latter under significant epigenetic control [15]. *KIR* genes are inherited in haplotypes of vastly diverse content, ranging from 4 to 14 receptor-encoding loci, with >50 distinct haplotypes based on gene content alone [13, 16]; approximately 700 allelic variants have been reported [16, 17]. Group 1 HLA-C (*HLA-C1*) allotypes have an asparagine at residue 80 and are ligands for the inhibitory receptors encoded by *KIR2DL2* and *KIR2DL3*, which segregate as alleles of a single locus, and KIR2DS2 [18]. The remaining HLA-C allotypes (group 2; *HLA-C2*) have a lysine at position 80 and are ligands for KIR2DL1 (an inhibitory receptor) and KIR2DS1 (the homologous activating receptor). HLA-B Bw4 allotypes serve as ligands for KIR3DL1 and KIR3DS1 (Figure 1).

The immunological mechanisms involved in clearance of HPV are poorly understood. NK cells are crucial for clearing viral infection and for antitumor immunity and are thought to be important in HPV control [19]. Regulation of NK cell responses depend on *KIR* genotype, *HLA* genotype, heterozygosity versus homozygosity for each of these, interaction of *HLA* and *KIR*, and changes in KIR and HLA expression. The development of imputation-based methods for *HLA* typing and, more recently, for *KIR* typing has enabled the use of single-nucleotide polymorphism (SNP)–typed data sets to investigate genetic associations with these loci in large cohorts. Here, *HLA* and *KIR* gene imputation and association tests were performed using genotype data from 2143 cervical neoplasia cases and 13 858 healthy controls of European descent to test whether *HLA-KIR* combinations are associated with cervical neoplasia risk.

METHODS AND MATERIALS

Study Population

Phenotypic information, including cervical histologic findings and HPV genotype, appears in Supplementary Table 1. As described by Leo et al [10], all cases needed to have cervical intraepithelial neoplasia types 2 and 3 (if type 2, an age of >30 years was required, to limit analysis to women who did not clear HPV) or cervical cancer (HPV type and histologic findings were not always known). HPV DNA types from tumor tissues were categorized into 6 groups: (1) HPV16 but not HPV18 positive, (2) HPV18 but not HPV16 positive, (3) neither HPV16 nor HPV18 positive, (4) both HPV16 and HPV18 positive, (5) negative for any HPV type (noting that usually samples are only tested for HPV16 and HPV18), and (6) those not tested for HPV genotype [10].

Genotyping

Case samples were SNP microarray genotyped in house. A total 791 cases were genotyped using Illumina OmniExpress BeadChips (Omni), and 1352 cases were genotyped using Illumina Human660-Quad BeadChips (660Q). Controls were genotyped using Illumina Immunochip BeadChips (Ichip; Figure 2). Bead intensity data were processed and normalized for each sample, and genotypes were called within participating studies using GenomeStudio and verified manually and corrected where necessary. Standard quality control measures were performed [10], with particular care taken in comparison of the performance of different chip types.

HLA Imputation

HLA alleles were inferred using HLA*IMP:03 (available at: http://www.biorxiv.org/content/early/2016/12/09/091009). Individuals with posterior probability of *HLA* alleles of

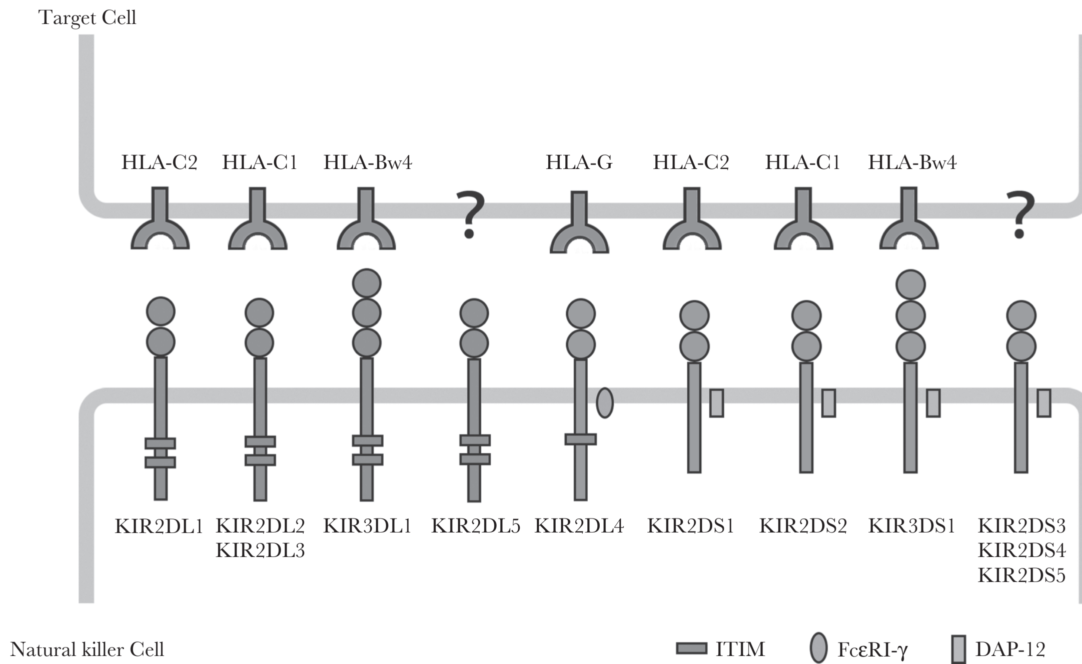

Figure 1. Killer immunoglobulin-like receptor (KIR) proteins are classified by the number of extracellular immunoglobulin domains (2D or 3D) and by whether they have a long (L) or short (S) cytoplasmic domain. Inhibitory KIRs and KIR2DL4 have immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domains. Activating KIRs possess a basic amino acid in the transmembrane domain, which allows interactions with the accessory molecule DAP-12, delivering activating signals through its immunoreceptor tyrosine-based activating (ITAM) motif. The ligands for several KIR proteins are subsets of HLA class I proteins. KIR2DL4 has a charged amino acid and ITIM motifs, and it interacts with the accessory protein FcεRI-γ, which sends an activating signal via its ITAM similar to DAP-12. Note that HLA-Bw6 alleles are not known to be KIR ligands.

<0.6 were excluded from downstream association testing (Supplementary Figure 1). *HLA* groups (*C1*, *C2*, *Bw4*, and *Bw6*) were inferred from known allele classifications. HLA amino acids were inferred by the SNP2HLA tool [20], using a reference panel from the Type 1 Diabetes Genetics Consortium (n = 5225). Amino acids imputed by SNP2HLA with an r^2 of <0.5 were excluded, and samples where the allele dosage at any HLA type was >2.5 were removed.

KIR Imputation

KIR genes and haplotypes were imputed with the KIR*IMP method [21], using SNP genotypes across the *KIR* locus. This requires certain informative key SNPs for accurate imputation, which vary according to the SNP chip used (Supplementary Table 2). To avoid imputation bias caused by different numbers of key SNPs genotyped by Omni and 660Q, the 2 groups of cases were imputed separately, with a separate comparator group for each obtained by randomly dividing the control group into 2 groups: Ichip control group 1 (Ichip1; n = 6703) and group 2 (Ichip2; n = 6725; Figure 2). Imputed data for *KIR* and *HLA* loci were compared between the 2 control groups. There were no significant differences between the 2 control groups for 271 *KIR* SNPs (because most of these were only available on Ichip [ie, not on chips used for case genotyping], there was a smaller number available for case-control analyses). Of 256 *HLA* alleles, 10 (3.9%) were significantly different ($P < .05$) between the 2 control groups. Most (8 of 10) were rare (≤0.05); the remaining 2 had a frequency of 0.08. None of these SNPs were significant in subsequent analyses. The 77 key SNPs for Omni cases and Ichip1 and the 66 key SNPs for 660Q cases and Ichip2 were reclustered manually (converting array intensity data for each allele of the SNP concerned to genotype calls) before imputation to improve genotyping accuracy (Supplementary Table 3). Individuals with a posterior probability of accurate *KIR* imputation for each *KIR* allele <0.6 were excluded in the downstream association testing.

Statistical Methods

Population stratification was assessed via principal component analysis of genome-wide genotypes, using Shellfish (available at: http://www.stats.ox.ac.uk/~davison/software/shellfish/shellfish.php). Association analyses were performed using custom R scripts. Three models were used to test *HLA-C1*, *HLA-C2*, *HLA-Bw4, HLA-Bw6*, and *HLA* alleles and their combination with *KIR* genes: (1) a dosage model, which treated genotypes as 0, 1, or 2 copies; (2) a dominant model, which treated 1 or 2 copy genotypes as present and 0 as absent; and (3) a recessive model to study the difference of homozygotes and heterozygotes of *HLA-B* and *HLA-C*, which treated 2 copy genotypes as present and 0 or 1 copy as absent. Meta-analysis of combination between

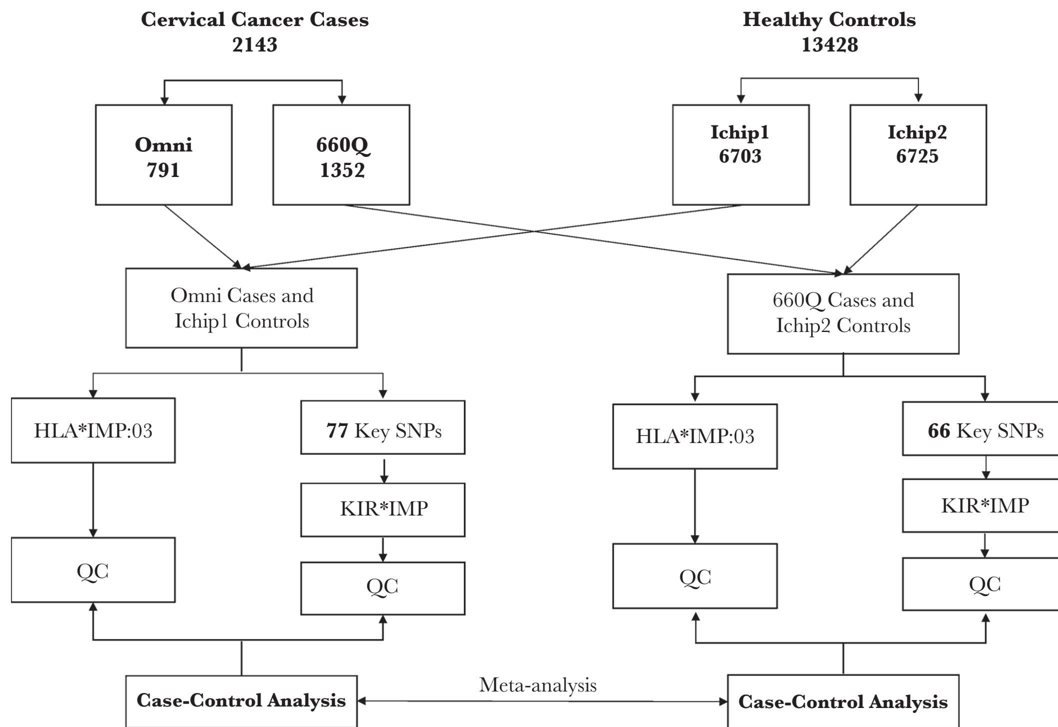

Figure 2. *HLA* and *KIR* gene imputation and association tests were performed using genotype data from 2143 cervical neoplasia cases and 13 858 healthy controls of European descent. A total of 791 individuals were genotyped using the Illumina OmniExpress BeadChip tool (Omni), and 1352 individuals were genotyped using the Illumina Human660-Quad BeadChip tool (660Q). All 13 428 controls were genotyped using the Illumina Immunochip BeadChip tool (Ichip). To avoid imputation bias caused by different numbers of key single-nucleotide polymorphisms (SNPs) between Omni and 660Q, these 2 groups of cases were imputed separately and compared to 2 control groups—Ichip control group 1 (Ichip1; n = 6703) and control group 2 (Ichip2; n = 6725)—obtained by randomly dividing the controls. A total of 77 key SNPs for Omni and Ichip1 and 66 key SNPs for 660Q and Ichip2 were reclustered and used for imputation. Case-control analyses were conducted separately, followed by meta-analysis. QC, quality control.

Omni-Ichip1 and 660Q-Ichip2 was performed using METAL software (available at: http://csg.sph.umich.edu/abecasis/metal/). For tests over all *HLA* alleles, the multiple testing correction method was used [22, 23], using a correlation matrix derived from SNP2HLA imputation with all 1027 *HLA* alleles and amino acids. This analysis estimated 206 independent loci, implying a Bonferroni-corrected *P* value of 2.4×10^{-4} for a type I error rate of 5%. For the *HLA-KIR* interaction using 12 *KIR* alleles, we used the most conservative Bonferroni-corrected *P* value (ie, $P = [0.05/(206 \times 12)] = 2.0 \times 10^{-5}$) for statistical significance.

RESULTS

Quality Control

A total of 2143 cases and 13 428 controls passed quality control. Among cases, 736 were squamous cell carcinoma, 542 were adenocarcinoma, and 865 had unspecified histologic findings. Four principal components were used as covariates to control for population stratification, calculated using 11 980 common SNPs shared by the Omni, 660Q, and Ichip microarrays. To assess population stratification, we used a subset of 333 null SNPs outside the MHC region included on each chip type and avoided SNPs included on the Ichip, because of their potential immunogenetic significance (ie, they are associated with reading and learning disability, schizophrenia, and psychosis). From this, the genomic inflation factor overall was calculated as 1.018 (Supplementary Figure 2). No divergences were observed between cases and controls or between different genotype platforms (Supplementary Figure 3). All *HLA* loci were imputed with an accuracy of >95% (Supplementary Table 4), and all *KIR* loci were imputed with an accuracy of >80% (Supplementary Table 3).

MHC Findings

HLA Association Analysis

Consistent with findings from our recent study [10], we found increased and decreased risks of cervical neoplasia associated with *HLA* haplotypes (Supplementary Table 5) and determined these associations were carried by amino acids at positions 13 and 71 in pocket 4 of HLA-DRB1 and at position 156 in HLA-B. Using these in-depth imputation methods across *HLA* and *KIR* loci, we detected further novel associations from *HLA-DRB3* and *HLA-DRB5*.

Novel risk associations with cervical neoplasia were identified with *HLA-DRB3*9901* (odds ratio [OR], 1.24; $P = 2.49 \times 10^{-9}$; Table 1) and *HLA-DRB5*0101* (OR, 1.29; $P = 2.26 \times 10^{-8}$). Although *HLA-DRB3*9901* was not in linkage disequilibrium with any individual cervical neoplasia *HLA* risk allele ($P < .05$),

Table 1. Analysis of Novel Cervical Neoplasia–Associated *HLA* Alleles, Conditioned on *HLA* Alleles and HLA Amino Acids

HLA Alleles	*DRB3*9901*	*DRB5*0101*	*DRB5*9901*	*DRB3*0301*
Unconditioned *P*	2.49×10^{-9}	2.26×10^{-8}	1.90×10^{-8}	4.06×10^{-6}
Odds ratio	1.24	1.29	0.77	0.63
Conditioned *P*				
*DRB3*9901*	NA	4.19×10^{-5}	3.66×10^{-5}	.001033
*DRB5*0101*	3.07×10^{-5}	NA	.45	4.43×10^{-5}
*DRB5*9901*	3.21×10^{-5}	.66	NA	4.50×10^{-5}
*DRB3*0301*	1.24×10^{-6}	8.7×10^{-8}	7.41×10^{-8}	NA
*DRB1*1501*	1.99×10^{-5}	.21	.37	5.28×10^{-5}
*B*0702*	9.64×10^{-6}	.0040	.0040	6.49×10^{-5}
*DQB1*0602*	6.94×10^{-6}	.0059	.0043	1.81×10^{-5}
*DRB1*1302*	3.18×10^{-6}	4.33×10^{-8}	4.45×10^{-8}	.14
DRB1_11	.0018	.055	.056	.0063
DRB1_13	4.73×10^{-6}	.01	.0096	.013
DRB1_37	.11	.01	.0092	.0063
DRB1_71	.013	.04	.037	.84
DRB1_96	.49	.00058	.0006	.001
DRB1_13 and *71*	2.26×10^{-5}	.37	.94	.54
DRB1_71 and *96*	.84	.046	.086	.7

Abbreviation: NA, not applicable.

adjustment for the association with HLA-DRB1 amino acid positions 37, 71, and 96 attenuated the association with *HLA-DRB3*9901* ($P > .01$). *HLA-DRB5*0101* was in positive linkage disequilibrium with the *HLA* class II risk allele *HLA-DRB1*1501* ($r^2 = 0.99$) and the protective allele *HLA-DRB5*9901* ($r^2 = 0.93$; Table 1 and Figure 3). No residual association for *HLA-DRB5*0101* was observed after control for the association of *HLA-DRB1*1501*, *HLA-DRB5*9901*, or *HLA-DRB1* amino acid positions 13 and 71 ($P < .01$).

Novel inverse associations with cervical neoplasia were observed with *HLA-DRB5*9901* (OR, 0.77; $P = 1.90 \times 10^{-9}$) and *HLA-DRB3*0301* (OR, 0.63; $P = 4.06 \times 10^{-5}$). *HLA-DRB5*9901* is in positive linkage disequilibrium with the HLA class II risk alleles *HLA-DRB1*1501* ($r^2 = 0.93$) and *HLA-DRB5*0101* ($r^2 = 0.93$; Table 1 and Figure 3). No residual associations at these 2 *HLA* loci were observed after control for the association of *HLA-DRB1* amino acid 71 ($P < .01$; Table 1).

HLA alleles were associated with disease when assessing HPV genotype without regard to histologic classification. Comparison of HPV16-related cases (n = 667) to all controls (n = 13 428) revealed risk associations with *HLA-DRB3*9901* (OR, 1.43; $P = 1.27 \times 10^{-8}$; Supplementary Table 6). Control for the association with HLA-DRB1 amino acid positions 13 and 37 controlled for the association of *HLA-DRB3*9901* ($P > .01$). For cases of HPV18-related cervical neoplasia (n = 166), the strongest associated *HLA* allele was *HLA-DPA1*0103* (OR, 1.89; $P = .00039$; Supplementary Table 7). With regard to histopathologic findings, a reduced risk was seen between *HLA-DRB5*9901* and squamous cell carcinoma (OR, 0.72; $P = 6.57 \times 10^{-5}$). No residual association was observed after control for the association of HLA-DRB1 amino acids 13, 17, and 96 ($P < .01$). An increased risk was seen between *HLA-DRB3*9901* and adenocarcinoma (OR, 1.31; $P = 7.38 \times 10^{-5}$; Supplementary Table 8). No residual association was observed after control for the association of HLA-DRB1 amino acids 13, 71, and 96 ($P < .01$).

HLA-Bw4/Bw6 and HLA-Cw1/Cw2 Association Analysis

Dominant and dosage models showed nominal risk associations between *HLA-Bw4* (OR, 1.24; $P = .014$) and *HLA-Bw6* (OR, 1.16; $P = .011$) and HPV16-related cervical neoplasia (Table 1 and Supplementary Table 9). The dosage model suggests that *HLA-Bw4* has a weak inverse association with HPV18-related cervical neoplasia (OR, 0.78; $P = .04$). No associations were found between *HLA-Bw4* or *HLA-Bw6* and cervical cancer overall, squamous cell carcinoma, or adenocarcinoma.

All 3 models suggested that *HLA-C1/2* alleles are associated with HPV16-related cervical neoplasia (Supplementary Table 10). *HLA-C1* and *HLA-C2* are mutually exclusive: an individual can have 2 copies of either or 1 copy of each. Both HLA-C1 and HLA-C2 can interact with specific KIRs and lead to inhibitory or activating signaling. To investigate the role of *HLA-C1* or *HLA-C2* and their combination with *KIR*, we used a recessive model to distinguish *HLA-C* homozygotes and heterozygotes. The frequency of individuals with 2 copies of *HLA-C1* alleles was lower in HPV16-related cervical neoplasia cases (35.7%) than controls (41.3%; OR, 0.79; $P = .005$; Table 2). The frequency of 2 copies of *HLA-C2* alleles did not differ between the groups. No association was found between *HLA-C1* or *HLA-C2* and cervical cancer overall, HPV18-related cervical cancer, squamous cell carcinoma, or adenocarcinoma.

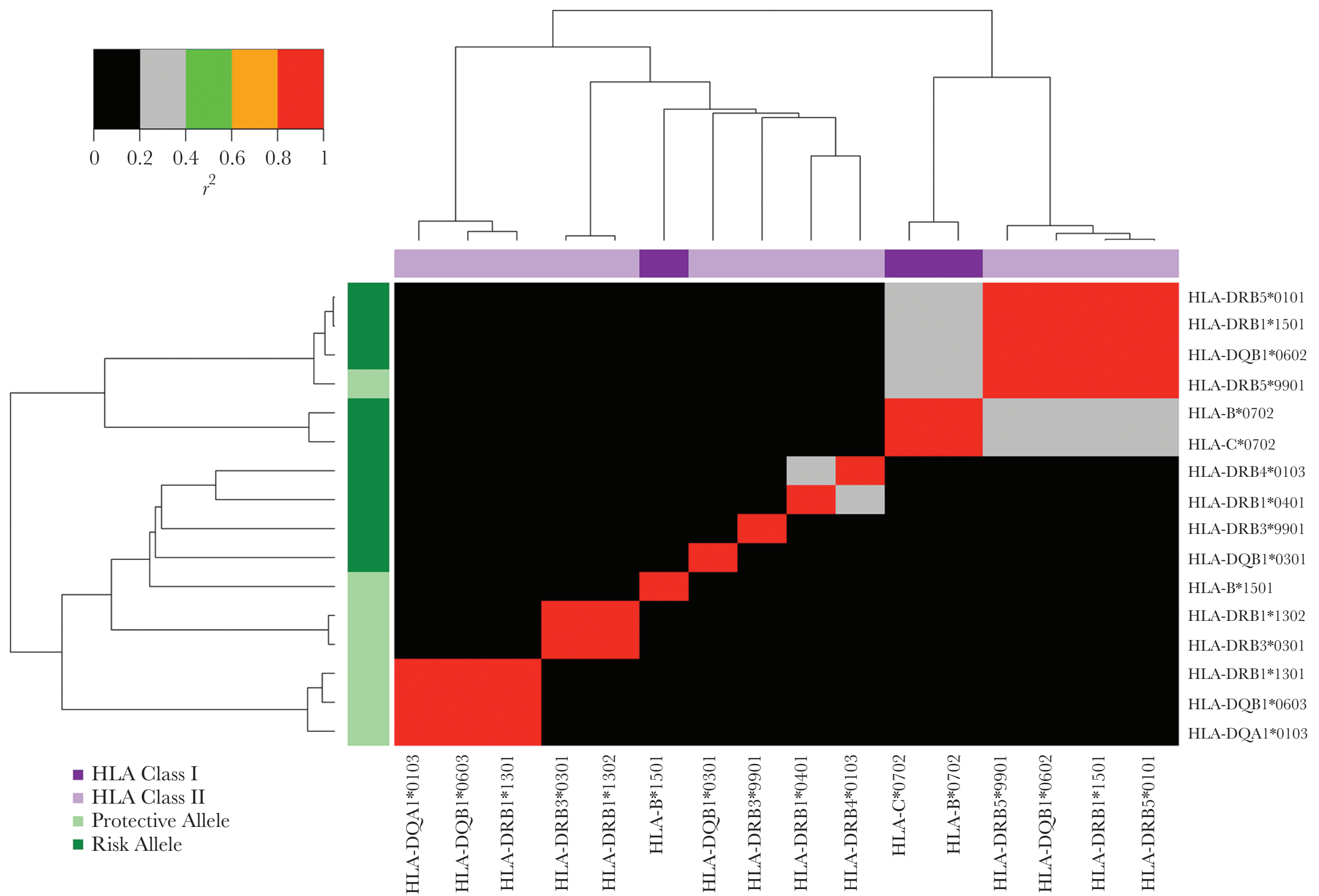

Figure 3. Pair-wise linkage disequilibrium (r^2) plot of *HLA* alleles associated with cervical neoplasia. *HLA* alleles are clustered according to their pair-wise linkage disequilibrium on both the *x*-axis and *y*-axis. On the *y*-axis, alleles are labeled with regard to whether they are risk or protective alleles in the overall cervical neoplasia data set, and on the *x*-axis, they are labeled according to whether they are HLA class I or II alleles.

KIR Findings

KIR imputation concordance was explored by comparing *KIR* haplotype frequencies among 4 data sets (Supplementary Figure 4), comparing published population prevalences (Supplementary Figure 5), and a control-control association test (Ichip1 vs Ichip2, using the randomly divided Ichip data set; Supplementary Table 11). For the control-control association test of 16 imputed *KIR* loci, 4 (*KIR2DS3, KIR2DL1, KIR2DP1*, and *KIR2DL5*) were inconsistent because of the different key SNP numbers in each group and were not analyzed further. Prevalences of the other 12 *KIR* genes were concordant between groups. No association was found between *KIR* genes and cervical cancer overall or HPV18-related cervical cancer. A weak protective association was seen between *KIR2DL2* and HPV16-related cervical cancer (OR_{omni}, 0.87; OR_{660Q}, 0.83; P_{meta} = .04).

KIR-HLA-Bw4/Bw6 and KIR-HLA-C1/2 Combinations

No *KIR-HLA-Bw4/Bw6 or KIR-HLA-C1/2* combination was associated with cervical neoplasia, HPV18-related cervical neoplasia, squamous cell carcinoma, or adenocarcinoma. *HLA-Bw4* alleles were associated with an increased risk of HPV16-associated cervical neoplasia; this association was restricted to *KIR3DL1* carriers (OR, 1.22; P_{meta} = .0085). No association was seen in individuals presenting with Bw6 *HLA-B* alleles.

KIR2DL3 and KIR2DL2 bind HLA-C1 allotypes, with KIR2DL2 binding with greater affinity [18]. The protective association for HPV16-related cervical neoplasia of *HLA-C1/C1* was restricted to individuals carrying *KIR2DL2* (P_{meta} = 0.00045; OR, 0.67), or *KIR2DS2* (P_{meta} = 0.0006; OR, 0.69), these *KIR* alleles often being found together on *KIR* haplotypes. In our dataset, *KIR2DL2* and *KIR2DS2* are in near complete linkage disequilibrium (r^2 = 0.99). *KIR2DL2* and *KIR2DL3* were not associated with cervical neoplasia in individuals who were lacking *HLA-C1/C1*. HLA-C2 can interact with either KIR2DS1 or KIR2DL1. No association with HPV16-related cervical neoplasia was seen in individuals with *KIR2DS1* and *HLA-C2*. *KIR2DL1* could not be investigated in this study due to the low *KIR2DL1* imputation accuracy.

KIR and HLA Allele Combinations

The strongest associations of any combination of *KIR* and *HLA* alleles with cervical neoplasia involved *HLA-B*5501* and *KIR2DS2* (OR_{omni}, 0.61; OR_{660Q}, 0.19; P_{meta} = 5.97 × 10^{-5})

Table 2. ***KIR-HLA-Bw4/Bw6* and *KIR-HLA-C1/C2* Type Combinations Associated With Human Papillomavirus Type 16 (HPV16)–Related Cervical Neoplasia**

Genetic Factor	HPV16-Positive Cases, Proportion (%)	Controls, Proportion (%)	OR[a] (95% CI)	*P*[b]
HLA-C1/C1	232/649 (35.7)	5434/13 148 (41.3)	0.79 (.67–.93)	.005
HLA-C1/C2	326/649 (50.2)	6054/13 148 (46)	1.18 (1.01–1.38)	.039
HLA-C2/C2	91/649 (14.0)	1660/13 148 (12.6)	1.13 (.90–1.42)	.29
2DL2	295/633 (46.6)	6658/13 073 (50.9)	0.84 (.72–.99)	.04
2DL3	596/647 (92.1)	11942/13 188 (90.6)	1.22 (.91–1.63)	.25
2DS2	310/648 (47.8)	6751/13 167 (51.3)	0.87 (.74–1.02)	.08
2DL2-C1/C1	96/624 (15.4)	2754/12 932 (21.3)	0.67 (.54–.84)	.00045
C1/C1 in 2DL2+	96/291 (33.0)	2754/6587 (41.8)	0.69 (.51–.88)	.0029
C1/C1 in 2DL2-	131/333 (39.3)	2599/6345 (41.0)	0.93 (.75–1.17)	.56
2DL3-C1/C1	216/638 (33.9)	4890/13 044 (37.5)	0.85 (.72–1.01)	.09
2DL2-C1/C2	158/624 (25.3)	3027/12 932 (23.4)	1.11 (.92–1.33)	.29
2DL3-C1/C2	291/638 (45.6)	5432/13 044 (41.6)	1.18 (1.00–1.38)	.053
2DS2-C1/C1	101/639 (15.8)	2791/13 023 (21.4)	0.69 (.55–.85)	.0006
C1/C1 in 2DS2+	101/306 (33.0)	2791/6677 (41.8)	0.68 (.54–.87)	.0024
C1/C1 in 2DS2-	131/333 (39.3)	2599/6346 (41.0)	0.93 (.75–1.17)	0.56
2DS2-C1/C2	165/639 (25.8)	3068/13 023 (23.6)	1.13 (.94–1.35)	.23
2DS1-C2/C2	22/652 (3.4)	573/13 199 (4.3)	0.77 (.50–1.19)	.15
HLA-Bw4	406/623 (65.2)	7459/12 375 (60.3)	1.24 (1.04–1.46)	.014
3DL1-HLA-Bw4	388/623 (62.3)	7111/12 375 (57.5)	1.22 (1.03–1.44)	.0085
HLA-Bw4 in 3DL1+	388/601 (64.5)	7111/11 805 (60.2)	1.20 (1.02–1.43)	.034
HLA-Bw4 in 3DL1-	18/22 (81.8)	348/570 (61.1)	2.8 (.96–8.6)	.059
3DS1+HLA-Bw4	156/623 (25.0)	2773/12 371 (22.4)	1.16 (.96–1.39)	.09

Frequencies of *HLA-B, HLA-C,* and *KIR-HLA-B, C* combinations among HPV16-infected cervical cancer cases and healthy controls are shown. *HLA-C1C1* indicates 2 group 1 *HLA-C* alleles, *HLA-C2C2* indicates 2 group 2 *HLA-C* alleles, and *HLA-C1C2* indicates 1 of each. *HLA-Bw4* indicates 1 or 2 group *Bw4* alleles.

Abbreviations: CI, confidence interval; HLA-C1, HLA-C01, 03, 07, 08, 12, 13, 14, and 16 and HLA-B4601 and 7301; HLA-C2, HLA-C02, 04, 05, 06, 15, 17, and 18; HLA-Bw4, HLA-B05, 5102, 5103, 13, 17, 27, 37, 38, 44, 47, 49, 51, 52, 53, 57, 58, 59, 63, and 77; - negative; +, positive.

[a]A positive odds ratio (OR) indicates a protective association with HPV16 infection.

[b]*P* values were calculated by using the R code glm model with principle components 1–4; combination *P* values were calculated from a meta-analysis between Omni-Ichip1 and 660Q-Ichip2 data sets

and *HLA-B*5501* and *KIR2DL2* (OR_{omni}, 0.6; OR_{660Q}, 0.2; P_{meta} = .00013; Table 3 and Supplementary Figure 6). *HLA-B*5501* was not associated with cervical neoplasia independent of this interactive association with *KIR* genes.

DISCUSSION

Here, we report novel associations between cervical neoplasia and combinations of *HLA* and *KIR* alleles. We also extend our previous findings of protective and risk haplotypes associated with cervical neoplasia, demonstrating associations of *HLA-DRB3* and *HLA-DRB5* alleles with the disease, and we confirm that these are due to linkage disequilibrium with HLA-DRB1 amino acids [10]. In our previous genome-wide associated study, no significant association was noted at the leukocyte receptor complex on chromosome 19q13, but many SNPs at this locus failed quality control because the complex genetic structure of the locus leads to reduced accuracy of genotype calling by automated algorithms. In the current study, careful manual checking of genotype calling was performed, and *KIR* genotypes were imputed and analyzed in combination with *HLA* alleles.

We used imputation methods to infer *HLA* and *KIR* genotypes from SNP microarray data to perform one of the largest *HLA-KIR* association studies reported to date. By comparing our imputed *KIR* haplotype and *KIR* gene prevalences with published data generated using direct genotyping approaches, we confirmed that concordance between genotyped and imputed data is high. We further compared our imputation findings with direct *HLA* and *KIR* genotype data in 86 1000 genome study samples. The concordance for *HLA* genes in 2-digit and 4-digit resolution was 99.77% and 99.42%, respectively (data not shown). While we were able to impute 16 *KIR* genes, the imputation of 4 genes was inconsistent between our 2 cohorts and therefore was not considered. Of the remaining 12, 2 (*KIR3DP1* and *KIR2DL4*) are framework *KIR* genes present in all individuals, and thus association with disease cannot be calculated for these genes. For the remaining 10 *KIR* genes, we did not find differences between cases and controls in isolation but demonstrated suggestive associations in combination with specific *HLA* alleles.

We identified 3 new *HLA* allelic associations with cervical neoplasia, *HLA-DRB5*0101* and HLA-*DRB3*9901* as risk factors, and *HLA-DRB3*301* as a protective factor. *HLA-DRB3*, *HLA-DRB4*, and *HLA-DRB5* are paralogs of *HLA-DRB1*, with which they are in linkage disequilibrium, and their expression level is one fifth that of *HLA-DRB1* (RefSeq, July 2008). Consistent with the strong linkage disequilibrium across this

Table 3. *KIR-HLA* Combinations Are Associated With Cervical Neoplasia

Combination			OR		Sample Size		HLA Frequency		KIR Frequency		KIR+HLA+		KIR-HLA-		KIR+HLA-		KIR-HLA+	
HLA Allele	*KIR* Gene	*P*[a]	Omni-Ichip1	660Q-Ichip2	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
*HLA-B*5501*	*KIR2DS2*	5.97×10^{-5}	0.61	0.19	2019	12650	0.034	0.034	0.52	0.51	21	240	923	5994	1028	6230	47	186
*HLA-B*5501*	*KIR2DL2*	.00013	0.6	0.21	1983	12563	0.034	0.033	0.511	0.508	21	233	922	5993	993	6151	47	186
*HLA-DQB1*0601*	*KIR2DL3*	.0032	0.14	0.17	2062	12975	0.012	0.0097	0.905	0.905	17	118	188	1215	1850	11634	7	8
*HLA-DRB1*1502*	*KIR2DL3*	.0042	0.2	0.18	1920	12349	0.012	0.0104	0.908	0.908	16	119	170	1133	1727	11088	7	9
*HLA-B*3801*	*KIR2DS4TOTAL*	.0047	0.16	0.19	2056	12800	0.025	0.023	0.956	0.955	46	287	85	575	1919	11931	6	7
*HLA-B*3801*	*KIR3DL1ex4*	.0047	0.16	0.16	2055	12807	0.025	0.023	0.956	0.954	46	287	85	576	1918	11937	6	7
*HLA-B*3801*	*KIR3DL1ex9*	.0048	0.16	0.19	2053	12805	0.026	0.023	0.956	0.954	47	290	85	575	1915	11933	6	7
*HLA-DQA1*0103*	*KIR2DL3*	.0078	0.57	0.5	2078	13106	0.084	0.120	0.905	0.906	147	1422	170	1086	1733	10453	28	145

Data are no. of cervical neoplasia cases and healthy controls with the specified *KIR-HLA* combinations, unless otherwise indicated.

Abbreviations: OR, odds ratio; -, negative; +, positive.

[a]Combination *P* values were calculated from a meta-analysis between Omni-Ichip1 and 660Q-Ichip2 data sets; *P* values of each data set were calculated by using the R code glm model with principal components 1–4.

locus, the associations between the *HLA-DRB3* and *HLA-DRB5* alleles and cervical neoplasia in this study were due to linkage disequilibrium with *HLA-DRB1* alleles and amino acid variants.

Homozygosity of the HLA-C1 genotype group (*C1/C1*) overall was associated with protection from HPV16-related cervical neoplasia. This protective association was restricted to carriers of either *KIR2DL2* or *KIR2DS2*, suggesting that it operates through a KIR-mediated mechanism. A role for *HLA-C1* genotypes in cervical neoplasia is supported by previous studies, although the direction of association has not been consistent, and studies have generally had small sample sizes. A study of cervical cancer–affected parent-case trios showed that HLA-C1 was overtransmitted among women with invasive cervical cancer ($P = .04$), particularly in the subgroup infected with HPV16 or HPV18($P = .008$) [24]. A study using unrelated cases and controls of European ancestry showed that the frequencies of *HLA-C1/KIR2DL2* and *HLA-C1/KIR2DL3* pairs were decreased in patients with HPV-positive cervical lesions and increased in those infected with high-risk HPV types, compared with findings for individuals infected with low-risk HPV types [25]. While these studies are not definitive, they suggest involvement of *HLA-C1* group alleles in modulating the risk of cervical neoplasia, perhaps through their function as KIR ligands.

Several small candidate gene studies of the *KIR* locus and cervical cancer have been performed. Consistent with our findings, a Western Australian cohort study of 147 cases demonstrated weak associations between *KIR2DL2* and *KIR2DS2* and high-grade cervical intraepithelial neoplasia types 2 and 3 overall ($P = .046$ and $P = .049$, respectively) [26]. A study of Eastern US and Costa Rican individuals (196 cases and 330 controls) indicated that the frequency of *KIR3DS1* increased and that of *HLA-C2* alleles decreased the risk of cervical cancer [27]. Our data do not support these findings. Other studies from Sweden (65 cases) [28], Brazil (79 cases) [29], and Korea (132 cases) [30] have not reported positive associations, although their samples sizes were too small to identify anything other than essentially monogenic risk associations.

The strongest *HLA-KIR* combination association with cervical neoplasia observed in this study was between *HLA-B*5501* and *KIR2DL2/DS2* ($P_{meta} = 5.97 \times 10^{-5}$), although this did not meet the conservative Bonferroni-corrected significance threshold of a *P* value of 2.0×10^{-5} for *HLA-KIR* interactions. While the frequency of *HLA-B*5501* did not differ between cases (3.5% [71 of 2015]) and controls (3.4% [426 of 12 458]), suggestive associations involving the combination of *HLA-B*5501* and either *KIR2DS2* or *KIRSDL2* were observed. The signal was mainly attributable to the 660Q-Ichip2 data. No imputation bias was found in *KIR2DS2*, *KIR2DL2*, and *HLA-B*5501* between case-case and control-control tests, suggesting that this was not an artifact of imputation, but in the absence of clear replication, further studies are required to determine its significance. *HLA-B55* has been reported to be associated with cervical neoplasia,

but this has not been replicated in larger studies [31]. KIR2DS2 and KIR2DL2 bind a range of HLA-C1 group allotypes, which include HLA-B46 and HLA-B73 but not HLA-B55. HLA-B55 is encoded by an *HLA-Bw6* allele, which does not bind KIR. It is possible that the association we observed here is due to linkage disequilibrium with other HLA types, or it may be a noninformative suggestive association, particularly given that it was only found in comparison with one of the 2 imputation sets.

Cervical neoplasia may be associated with *HLA-C1* group alleles that interact with *KIR* in controlling NK cell activation. Further HLA associations were demonstrated and shown to be driven by linkage disequilibrium with known amino acid components of HLA-DRB1 allelic associations of disease, further supporting that these variants are key to HLA-associations of cervical neoplasia. No definitive *KIR* associations were noted with cervical neoplasia, although we only examined *KIR* gene carriage and not allelic variation within *KIR* genes, for which larger and more densely sequenced reference data sets for imputation will be required. Further studies of *KIR* allelic variation are warranted, particularly given the role of KIR-bearing cells in immunity to viral infection and the suggestive association of *HLA-C1* variants observed with disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank Jessica Darlington-Brown, for coordinating specimen collection for the New South Wales, Australia, component; and Dr David Pennisi, for proofreading and copyediting the manuscript.

Financial support. This work was supported by the National Health and Medical Research Council, Australia (senior principal research fellowship to M. A. B. and grant 387701); the Australian Cancer Research Foundation; the National Cancer Institute, National Institutes of Health (grants P01CA042792 and R01CA112512); the Cancer Council New South Wales, Australia; the Canadian Institutes of Health Research (grant MOP-42532); Réseau sida et maladies infectieuses du Fonds de recherche du Québec – Santé; the Swedish Research Council; the Swedish Foundation for Strategic Research; the LUA-ALF (research grants via the University of Gothenburg and the University of Umeå); the Lundberg Foundation; the Torsten and Ragnar Söderberg's Foundation; the Novo Nordisk Foundation; the European Commission (grant HEALTH-F2-2008-201865-GEFOS, BBMRI.se); the Swedish Society of Medicine; the Kempe-Foundation (grant JCK-1021); the Medical Faculty of Umeå University; the County Council of Västerbotten (grant Spjutspetsanslag VLL:159:33–2007); the Canada Research Chairs Program (tier 1 Canada research chair in human genome epidemiology to J. L.); and the Royal Women's Hospital Clinical Research Foundation.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Ferlay JSI, Ervik M, Dikshit R, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase no. 11. http://globocan.iarc.fr. Accessed 8 September 2017.
2. Fidler MM, Gupta S, Soerjomataram I, Ferlay J, Steliarova-Foucher E, Bray F. Cancer incidence and mortality among young adults aged 20–39 years worldwide in 2012: a population-based study. Lancet Oncol **2017**; 18:1579–89.
3. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30 848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer **2011**; 128:927–35.
4. Dunne EF, Park IU. HPV and HPV-associated diseases. Infect Dis Clin North Am **2013**; 27:765–78.
5. Stewart BW, Wild C, International Agency for Research on Cancer, World Health Organization. World cancer report 2014. Lyon, France Geneva, Switzerland: International Agency for Research on Cancer WHO Press, **2014**.
6. Schiffman M, Glass AG, Wentzensen N, et al. A long-term prospective study of type-specific human papillomavirus infection and risk of cervical neoplasia among 20 000 women in the Portland Kaiser Cohort Study. Cancer Epidemiol Biomarkers Prev **2011**; 20:1398–409.
7. Ramoz N, Rueda LA, Bouadjar B, Montoya LS, Orth G, Favre M. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. Nat Genet **2002**; 32:579–81.
8. Hernandez PA, Gorlin RJ, Lukens JN, et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat Genet **2003**; 34:70–4.
9. Lesseur C, Diergaarde B, Olshan AF, et al. Genome-wide association analyses identify new susceptibility loci for oral cavity and pharyngeal cancer. Nat Genet **2016**; 48:1544–50.
10. Leo PJ, Madeleine MM, Wang S, et al. Defining the genetic susceptibility to cervical neoplasia-A genome-wide association study. PLoS Genet **2017**; 13:e1006866.

11. Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease, 5th edition. New York: Garland Science; **2001**.
12. Martin AM, Freitas EM, Witt CS, Christiansen FT. The genomic organization and evolution of the natural killer immunoglobulin-like receptor (KIR) gene cluster. Immunogenetics **2000**; 51:268–80.
13. Pyo CW, Wang R, Vu Q, et al. Recombinant structures expand and contract inter and intragenic diversification at the KIR locus. BMC Genomics **2013**; 14:89.
14. Fauriat C, Ivarsson MA, Ljunggren HG, Malmberg KJ, Michaëlsson J. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. Blood **2010**; 115:1166–74.
15. Schenk A, Bloch W, Zimmer P. Natural killer cells–an epigenetic perspective of development and regulation. Int J Mol Sci **2016**; 17:326.
16. Norman PJ, Hollenbach JA, Nemat-Gorgani N, et al. Defining KIR and HLA class I genotypes at highest resolution via high-throughput sequencing. Am J Hum Genet **2016**; 99:375–91.
17. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SG. The IPD and IMGT/HLA database: allele variant databases. Nucleic Acids Res **2015**; 43:D423–31.
18. Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. J Immunol **1998**; 161:571–7.
19. Villa LL. Human papillomaviruses and cervical cancer. Adv Cancer Res **1997**; 71:321–41.
20. Jia X, Han B, Onengut-Gumuscu S, et al. Imputing amino acid polymorphisms in human leukocyte antigens. PLoS One **2013**; 8:e64683.
21. Vukcevic D, Traherne JA, Næss S, et al. Imputation of KIR types from SNP variation data. Am J Hum Genet **2015**; 97:593–607.
22. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity (Edinb) **2005**; 95:221–7.
23. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet **2004**; 74:765–9.
24. Martin MP, Borecki IB, Zhang Z, et al. HLA-Cw group 1 ligands for KIR increase susceptibility to invasive cervical cancer. Immunogenetics **2010**; 62:761–5.
25. Rizzo R, Gentili V, Rotola A, Bortolotti D, Cassai E, Di Luca D. Implication of HLA-C and KIR alleles in human papillomavirus infection and associated cervical lesions. Viral Immunol **2014**; 27:468–70.
26. Brestovac B, Wong ME, Tjendera R, Costantino PJ, Mamotte C, Witt CS. Human papillomavirus, high-grade intraepithelial neoplasia and killer immunoglogulin-like receptors: a Western Australian cohort study. Infect Agent Cancer **2013**; 8:33.
27. Carrington M, Wang S, Martin MP, et al. Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci. J Exp Med **2005**; 201:1069–75.
28. Arnheim L, Dillner J, Sanjeevi CB. A population-based cohort study of KIR genes and genotypes in relation to cervical intraepithelial neoplasia. Tissue Antigens **2005**; 65:252–9.
29. Marangon AV, Guelsin GA, Visentainer JE, et al. The association of the immune response genes to human papillomavirus-related cervical disease in a Brazilian population. Biomed Res Int **2013**; 2013:146079.
30. Song MJ, Lee CW, Kim JH, et al. Association of KIR genes and HLA-C alleles with HPV-related uterine cervical disease in Korean women. Tissue Antigens **2013**; 81:164–70.
31. Krul EJ, Schipper RF, Schreuder GM, Fleuren GJ, Kenter GG, Melief CJ. HLA and susceptibility to cervical neoplasia. Hum Immunol **1999**; 60:337–42.