Killer cell immunoglobulin-like receptors (KIR) and human leukocyte antigen-C (HLA-C) allore cognition patterns in women with endometriosis

Ya-ching Chou1,2,3,4, Chi-Huang Chen1,2, Ming-Jer Chen5,6, Ching-Wen Chang1, Pi-Hua Chen7,8, Mu-Hsien Yu9, Yi-Jen Chen10,11, Eing-Mei Tsai12,13, Peng-Sheng Yang1, Shyr-You Lin1,2 & Chii-Ruey Tzeng1,2*

Endometriosis shares similarities with several autoimmune diseases. The human leukocyte antigen (HLA)-C genotype is associated with several human autoimmune diseases. HLA-C is a ligand of killer cell immunoglobulin receptors (KIRs) and is an essential regulator of natural killer cell activity, which is associated with endometriosis progression. Polymorphisms in HLA-C and KIR affect the activity of NK cells and susceptibility to several diseases. Therefore, we attempted to investigate an association between HLA-C genotype and KIR polymorphism and the occurrence of endometriosis. We tested the association of certain KIR and HLA-C combinations and the development of endometriosis by characterizing both KIR and HLA-C genes in 147 women with endometriosis and 117 controls. The HLA-C genotypes and KIR polymorphisms were analyzed via DNA-based method for higher-resolution genotyping. We found that the occurrence of HLA-C*03:03*01 was increased in endometriosis than in control groups. Analysis of various KIR haplotypes revealed differences between the endometriosis and control cohorts. The number of KIR centromeric A/A haplotypes was increased in the endometriosis group than controls. Moreover, the endometriosis cohort was characterized by reduced number of KIR2DS2-positive individuals in the Han Chinese population. Our current findings suggest that the KIR and HLA-C genotypes are associated with the pathogenesis of endometriosis.

Endometriosis is a chronic gynecological disease with unknown etiology and is characterized by extra-uterine growth of endometrial tissue1. Endometriosis affects 6% to 10% of fertile women at the reproductive age and causes severe pelvic pain and infertility2–4. Familial and twin studies have reported that genetic factors are associated with the pathogenesis of endometriosis5–9. Cell-mediated and humoral immune responses are essential in the pathogenesis of endometriosis, since it is associated with various immunological abnormalities, particularly

1Center for Reproductive Medicine & Sciences, Department of Obstetrics and Gynecology, Taipei Medical University Hospital, Taipei, Taiwan. 2Department of Obstetrics and Gynecology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. 3Department of Biological Science and Technology, College of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan. 4Center for Intelligent Drug Systems and Smart Bio-devices (IDS²B), National Chiao Tung University, Hsinchu, Taiwan. 5Department of Obstetrics and Gynecology and Women’s Health, Taichung Veterans General Hospital, Taichung, Taiwan. 6School of Medicine, National Yang-Ming University, Taipei, Taiwan. 7Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. 8Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, Taipei, Taiwan. 9Department of Obstetrics & Gynecology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan. 10Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, Taipei, Taiwan. 11School of Medicine, Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan. 12General Research Centers of R&D office, Kaohsiung Medical University, Kaohsiung, Taiwan. 13Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

*email: tzengcr@tmu.edu.tw
significant.

**Table 1.** Patient demographic results. Abbreviations: BMI, body mass index; SD, standard deviation Mean (SD) for continuous variables. n (%) for discontinuous variables. *Mann-Whitney test. **χ**²-test.

| Characteristics                  | Control n = 117 (%) | Endometriosis n = 147 (%) | p value |
|----------------------------------|---------------------|---------------------------|---------|
| Agea                             | 38.44 (7.47)        | 36.08 (6.55)              | 0.012   |
| BMI, kg/m²                       | 23.01 (4.47)        | 21.58 (3.47)              | 0.0032  |
| Age of menarchea                 | 12.53 (1.21)        | 12.80 (1.52)              | 0.5968  |
| Duration of Menstrual cycleb     | 27.95 (4.95)        | 28.45 (2.97)              | 0.1392  |
| Dysmenorrheac, n (%)             | 73 (62.39)          | 112 (76.19)               | 0.015   |

Cell-mediated immunity10-12. The activities of cytotoxic T-cells and natural killer (NK) cells are dysregulated in women with endometriosis13-16. Increased serum levels of immunoglobulins and autoantibodies, decreased endometrial apoptosis, and the production of pro-inflammatory cytokines are observed in endometriosis patients, indicating that endometriosis shares many similarities with autoimmune diseases11,12,17,18.

Major histocompatibility complex (MHC) genes, also known as human leukocyte antigen (HLA) genes, are located in chromosome 6p. The genes encoding the human MHC class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DR, HLA-DQ, and HLA-DP) molecules are the most polymorphic loci in the human genome. HLA genes are polymorphic in binding and function in presenting antigen peptides to T-cells. HLA molecules are key factors involved in regulating the specificity of T-cell-mediated immune response in autoimmune and infectious diseases19-21.

**HLA** Class I genes encode cell-surface proteins, whose primary functions are to present antigens to cytotoxic CD8⁺ T-cells during the early immune responses19-21. Among these, HLA-C plays a minor role in regulating antigen-specific T-cell responses because of low cell surface expression22. HLA-C acts as a ligand for killer cell immunoglobulin-like receptors (KIRs), which regulate natural killer (NK) cell-mediated cytotoxicity. The human immunodeficiency virus Nef protein selectively downregulates the production of HLA-A and HLA-B molecules to suppress cytotoxic CD8⁺ T lymphocyte responses23. Importantly, the HLA-C genotype has been implicated in several autoimmune diseases, including Graves’ disease, psoriasis, and Crohn’s disease20,22,23,25,26.

NK cells are lymphocytes that serve as vital components of the immune system by regulating early responses against infected or transformed cells via cytokine production and direct cytotoxicity27. KIRs are a family of membrane glycoproteins expressed by NK cells. KIRs contain two or three extracellular immunoglobulin-like domain molecules (D) with a long (L) or short (S) cytoplasmic tail. The KIR gene is located on chromosome 19q13.4 on the leucocyte receptor complex. KIR exhibits activating and inhibitory effects with extensive haplotypic and allelic polymorphisms29-31. The 16 KIR genes comprise the following six genes encoding activating KIR (2DS1-5 and 3DS1), seven genes encoding inhibitory KIR (2DL1-3, 5 and 3DL1-2), KIR2DL4, which can exert both inhibitory and activating activity, and two pseudogenes (2DP1 and 3DP1). Furthermore, KIR3DL3, KIR3DP1, KIR2DL4, and KIR3DL2 are framework genes and are always present in the genome32.

The primary ligands of KIR are HLA-C molecules, which are divided into two groups, namely C1 and C2, based on the amino acid at position 80 [HLA-C C1 groups (HLA-C1), asparagine (N) at position 80: C*01, 03, 07 (01-06), 08, 12 (02, 03, 06), 13, 14, 15:07, 16 (01, 03, 04); HLA-C C2 groups (HLA-C2), lysine (K) at position 80: C*02, 04, 05, 06, 07 (07), 12 (04, 05, 42), 15, 16, 02, 17, 18)]32,33. The inhibitory receptors KIR2DL2 and KIR2DL3 and activating receptor KIR2DS2 share the same ligand HLA-C1. Activating KIR2DS2 has been reported to be in strong linkage disequilibrium and highly homologous to KIR2DL2. KIR2DL1 and KIR2DS1 bind to HLA-C23134-36. Combinations of HLA-C with KIR2DS1 and KIR2DS2 have been reported to correlate with the occurrence of autoimmune diseases, leukemia, and inflammatory diseases37-42. Polymorphisms in the genes encoding HLA-C and KIR affect NK cell activity and susceptibility to several diseases41. HLA genotyping is traditionally performed using a serological method. However, detection of the HLA-C genotype via serological typing is difficult because of the low HLA-C expression levels at the cell surface, the lack of suitable antisera, and difficulties in protein isolation42. Therefore, we employed a DNA-based method for higher-resolution genotyping and investigated the association between the HLA-C genotype and endometriosis. Moreover, to analyze the association between certain KIR-HLA-C combinations and the development of endometriosis, we characterized both KIR and HLA-C gene polymorphisms in 147 women with endometriosis and 117 controls.

**Results**

**Frequency distributions of HLA-C alleles among endometriosis and control groups.** The demographic results of endometriosis and control groups are shown in Table 1. HLA-C allele frequencies in endometriosis patients (n = 147, 294 alleles) and control patients (n = 117, 234 alleles) were determined using a sequence-based typing method. The presence of HLA-C*03:03:01 significantly increased the risk of endometriosis with p = 0.0473 [Odds Ratio (OR) = 2.811, 95% confidence interval (CI) = 1.021–7.738] and the statistical power was 43.8% (Table 2). After multiple tests analyses using Bonferroni correction, the association was not significant.

**Frequency distributions of HLA-C group among endometriosis and control groups.** We evaluated whether the HLA-C group C1 (HLA-C1) and HLA-C group C2 (HLA-C2) were associated with...
endometriosis. Analysis revealed no significant differences in HLA-C1 and HLA-C2 frequencies in the endometriosis and control groups (Table 3).

Using sequence-specific PCR amplification, we analyzed the KIR genotypes in the endometriosis and control groups. The frequencies of the KIR genotypes in women with endometriosis and controls and their statistical associations are presented in Table 4. The presence of KIR2DS2 significantly reduced the risk of endometriosis with \( \text{OR} = 0.5577, 95\%\ CI = 0.3251–0.9569 \) and the statistical power was 68.6%. After multiple test analyses using Bonferroni correction, the association was not significant. The two groups showed no significant differences in the remaining KIR genotypes.

### Table 2. Distribution of the HLA-C alleles in the endometriosis and control groups. Each HLA allele has four unique sets denoted by different numbers that are separated by a colon. The first two digits often correspond to the serological antigen; the two digits after the first colon denote the subtypes and order in the genome from the IMGT/HLA Database (www.ebi.ac.uk/imgt/hla/). The differences in HLA-C allele frequencies between the endometriosis and control groups were analyzed using the Fisher's exact test. Significance was set at a P value < 0.05 and the statistical power was 43.8% calculated by G*Power. OR indicates odds ratio. CI indicates confidence interval.

| HLA-C | Control (n = 117, 234 alleles) | Endometriosis (n = 147, 294 alleles) | OR | 95% CI | P value |
|-------|-------------------------------|-------------------------------------|----|--------|---------|
| C1    | 146 99.3                       | 146 99.3                           | 2.539 | 0.2273 to 28.37 | 0.5859 |
| C2    | 36 24.5                        | 36 24.5                            | 0.9842 | 0.5602 to 1.729 | 1 |
| C1:C2 | 111 75.2                       | 111 75.5                           | 1.016 | 0.5783 to 1.785 | 1 |
| C2:C2 | 1 0.7                          | 1 0.7                              | 0.3938 | 0.03525 to 4.400 | 0.5859 |
| C1:C2 | 35 23.8                        | 35 23.8                            | 1.042 | 0.5869 to 1.849 | 1 |

### Table 3. Distribution of HLA-C ligand in endometriosis and control groups. Two-sided Fisher’s exact test was used to estimate the differences between endometriosis and control groups. n: number of cases with relevant genotypes, OR: odds ratio, CI: confidence interval, Significance was set at a P value < 0.05.

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**Frequency distributions of KIR genotypes among endometriosis and control groups.** Using sequence-specific PCR amplification, we analyzed the KIR genotypes in the endometriosis and control groups. The frequencies of the KIR genotypes in women with endometriosis and controls and their statistical associations are presented in Table 4. The presence of KIR2DS2 significantly reduced the risk of endometriosis with \( \text{OR} = 0.5577, 95\%\ CI = 0.3251–0.9569 \) and the statistical power was 68.6%. After multiple test analyses using Bonferroni correction, the association was not significant. The two groups showed no significant differences in the remaining KIR genotypes.
eral human autoimmune diseases. HLA-C, HLA-DQB1, and HLA-DRB1 are involved in the pathogenesis of endometriosis. In a previous study, PCR-restriction fragment length polymorphism analysis revealed that the HLA-C*03:01 allele variant. Two-sided Fisher’s exact test was used to estimate the differences between endometriosis and control groups. n: number of cases with relevant genotypes, OR: odds ratio, CI: confidence interval. *versus controls, p < 0.05 and the statistical power was 68.6% calculated by G*Power.

| Inhibitory KIR | Control (n = 117) | Endometriosis (n = 147) | OR | 95% CI | p value |
|---------------|------------------|-------------------------|----|--------|---------|
| KIR2DL1       | 114              | 147                     | 100.0 | 9.017  | 0.4608 to 176.5 | 0.0858 |
| KIR2DL2       | 37               | 31                      | 21.1 | 0.5778 | 0.3314 to 1.007 | 0.0653 |
| KIR2DL3       | 114              | 146                     | 99.3 | 3.842  | 0.3942 to 37.45 | 0.3252 |
| KIR2DL4       | 117              | 147                     | 100.0 | —      | —       | — |
| KIR2DL5       | 52               | 44                      | 33.3 | 0.625  | 0.3788 to 1.031 | 0.0747 |
| KIR3DL1       | 115              | 98.3                    | 98.6 | 1.261  | 0.1748 to 9.093 | 1 |
| KIR3DL2       | 117              | 100.0                   | 100.0 | —      | —       | — |
| KIR3DL3       | 117              | 100.0                   | 100.0 | —      | —       | — |

Table 4. Genetic association between KIR gene in endometriosis and control groups. The gene was considered positive if either of the two forms were present. KIR2DS4f - full-length KIR2DS4 allele variant. KIR2DS4d - deleted KIR2DS4 allele variant. Two-sided Fisher’s exact test was used to estimate the differences between endometriosis and control groups. n: number of cases with relevant genotypes, OR: odds ratio, CI: confidence interval, *versus controls, p < 0.05 and the statistical power was 68.6% calculated by G*Power.

Frequency distributions of KIR haplotypes among endometriosis and control groups. The frequencies of the KIR haplotypes in women with endometriosis and controls and their statistical associations are presented in Table 5. The \( \chi^2 \) value was calculated by Hardy-Weinberg analysis (\( \chi^2 > 3.841 \) showed the subgroup was deviating from the Hardy–Weinberg equilibrium). We revealed differences between the endometriosis and control cohorts. The number of KIR centromeric A/A haplotypes was increased in the endometriosis group than controls with \( p = 0.0394 \) ([OR] = 1.793, 95% CI = 1.045–3.076) and the statistical power was 68.6%.

Combination of KIR and their HLA-C ligands. The frequencies of KIR and their HLA-C ligands were analyzed for their statistical associations with endometriosis (Table 6). HLA-C C1 groups are recognized by KIR2DL2/2DS2 and KIR2DL3, while HLA-C C2 groups are recognized by KIR2DL1/2DS1. Moreover, KIR2DL2/2DL3 also binds to some HLA-C C2 molecules, and KIR2DS4 binds to some HLA-C1 and HLA-C2. The molecular interactions of KIR gene-HLA-ligands were calculated from the KIR frequency of a total number of HLA ligands. The total number of HLA ligands is shown in Table 3. We calculated the KIR frequency in the combination of different HA ligands. Analysis of various KIR-HLA-C combinations revealed no significant differences between the endometriosis and control groups (Table 6). The frequency of KIR haplotypes and HLA‐C combinations also showed no significant differences between the endometriosis and control groups (Table 7).

Discussion

Several factors are involved in the pathogenesis of endometriosis including genetic, neuroendocrine, and immunological factors. Abnormal immune responses are recognized in endometriosis patients, including excessive inflammatory cytokine secretion, autoantibody production, and NK cell regulation. Endometriosis shares similar characteristics with autoimmune diseases. HLA-C affects viral infections and is implicated in several human autoimmune diseases. HLA-C*06:02 is associated with severe early-onset psoriasis. HLA-C*12:02 was found to be associated with increased susceptibility to Crohn's disease. HLA-C*03 restricts the cytotoxic CD8+ T-cell responses during the Epstein-Barr virus and human immunodeficiency virus infections, as well as during co-infection with the influenza virus and the Sendai virus. Herein, we analyzed the associations between HLA-C alleles and endometriosis. Consequently, the presence of HLA-C*03:03:01 increased the risk of endometriosis in Asian women (Table 2). However, after multiple test analyses using Bonferroni correction, the association was not significant.

Previous studies reported no association between HLA genotypes and endometriosis in Caucasian women with endometriosis and controls, as assessed by serological typing. A serological study showed increased frequencies of the HLA-B*54 and HLA-C*07 alleles in Japanese patients with endometriosis. In a recent study, PCR-restriction fragment length polymorphism analysis revealed that the HLA-DRB1*14:03 and HLA-DQBI*03:01 alleles are associated with endometriosis in Japanese women. Another study reported an
The association between the HLA-A*24, HLA-B*07:02, HLA-C*07:02, and HLA-DRB1*01:01 haplotypes and endometriosis in Japanese women has been investigated. A previous study showed that HLA-DRB1 alleles were not associated with endometriosis in Polish women. A literature search identified similar reports from China, which showed an association between endometriosis and the HLA-B*46, HLA-DRB1*15, and HLA-DQA1*0401 alleles. The reasons underlying the discrepancies observed among these studies are unclear; however, the results may have been influenced by differences in the ethnicities of the women in the study cohorts and the differences in the detection methods.

The frequency of KIR3DS1 was significantly lower in endometriosis patients compared to control patients. Moreover, the protective effect of the KIR2DS5 gene was observed in endometriosis patients. Nowak et al. showed that the protective effect of KIR2DS5 was present only in the women who harbored the HLA-C C2 group. Our current findings revealed that a lower proportion of endometriosis groups, which was characterized by the presence of activating KIR2DS2 compared to the control groups (Table 5).

Inhibitory KIR-ligand association

| KIR2DL1-HLA-C1/C2 | Control | Endometriosis | OR | 95% CI | p value |
|-------------------|---------|---------------|----|--------|---------|
| 27                | 100.0%  | 35            | 100.0%        | —     | —       |
| 1                 | 50.0%   | 1             | 100.0%        | 3      | 0.05947 to 151.3 | 1 |
| 25                | 28.4%   | 20            | 18.0%         | 0.5538 | 0.2834 to 1.083 | 0.0902 |
| 10                | 37.0%   | 11            | 31.4%         | 0.7792 | 0.2704 to 2.245 | 0.7876 |
| 86                | 97.7%   | 110           | 99.1%         | 2.558  | 0.2280 to 28.70 | 0.5847 |
| 26                | 96.3%   | 35            | 100.0%        | 4.019  | 0.1573 to 102.7 | 0.4355 |

Activating KIR-ligand association

| KIR2DS1-HLA-C1/C2 | Control | Endometriosis | OR | 95% CI | p value |
|-------------------|---------|---------------|----|--------|---------|
| 5                 | 18.5%   | 13            | 37.1%         | 2.6    | 0.7918 to 8.538 | 0.1593 |
| 2                 | 100.0%  | 0             | 0.0%          | 0.0667 | 0.00008081 to 5.5 | 0.3333 |
| 28                | 31.8%   | 23            | 20.7%         | 0.5601 | 0.2947 to 1.064 | 0.1016 |
| 11                | 40.7%   | 11            | 31.4%         | 0.6667 | 0.2337 to 1.902 | 0.5932 |

Table 5. Frequency of centromeric and telomeric KIR haplotypes in endometriosis and control groups. Two-sided Fisher's exact test was used to estimate the differences between endometriosis and control groups. n: number of cases with relevant genotypes, OR: odds ratio, CI: confidence interval, $\chi^2$ value was calculated by Hardy-Weinberg analysis ($\chi^2 > 3.841$ showed the subgroup was deviating from the Hardy–Weinberg equilibrium). *versus controls, $P < 0.05$ and the statistical power was 68.6% calculated by G*Power.

Table 6. Distribution of molecular interactions of KIR gene-HLA-ligands in endometriosis and control groups. The molecular interactions of KIR gene-HLA-ligands were shown in the frequency of the KIR gene of HLA-ligands, which was calculated from the KIR frequency of the total number of HLA ligands. The total number of HLA ligands is shown in Table 3. Two-sided Fisher's exact test was used to estimate the differences between endometriosis and control groups. n: number of cases with relevant genotypes, OR: odds ratio, CI: confidence interval.
The final activation status of functional NK cells depends on the homeostasis of all NK cell activation/inhibitory receptors and the corresponding ligands. Then, NK cells are regulated in endometrium in women with endometriosis. Further studies will be worth elucidating the functional relevance of the presence of these receptors and ligand proteins in the endometrium. The limitation of this study is that only the stage III or stage IV endometriosis patients were enrolled to investigate the genetic associations. Thus, our results did not show any association between genes and severity of disease. Further clinically relevant studies on the severity of disease and genetic associations are required.

Our current findings demonstrated the association between KIR polymorphisms and HLA-C genotypes with endometriosis in women. It is the first study addressing KIR polymorphisms and HLA-C genotypes of women with stage III or IV endometriosis in Han Chinese women. The results suggested that HLA-C and KIR genotypes influence the susceptibility for endometriosis. Further studies should investigate the role of NK cells in the pathogenesis of endometriosis.

Methods

Patients and controls. Han Chinese women were categorized into the endometriosis (n = 147) and control (n = 117) groups. Endometriosis was diagnosed via laparoscopic examination and confirmed via histological assessment. A total of 147 women were classified under stage III or IV endometriosis in accordance with the Revised American Society for Reproductive Medicine Classification. Women in the control group underwent benign gynecological surgery and showed no evidence of endometriosis including myoma, teratoma, serous cystadenoma, ovarian cyst, ovarian stroma, dermoid cyst, mucinous cystadenoma, paratubal cyst, follicular cyst, simple cyst, hydrosalpinx, corpus luteum cyst, fibrous adhesion, and struma ovarii. Considering that autoimmune disorders are associated with HLA-C alleles and KIR genotypes, the exclusion criteria comprised autoimmune disorders. The protocol was approved by the Institutional Review Board of the Taipei Medical University Hospital, and all participants have informed consent. All experiments were performed in accordance with relevant guidelines and regulations.

DNA extraction and HLA-C and KIR genotype analysis. Genomic DNA was extracted using a DNA whole-blood kit following the manufacturer's instructions (Kurabo Industries, Osaka, Japan). HLA-C was genotyped using an HLAssure™ SE sequence-based typing kit (TBG Biotechnology Corp, Queensland, Australia), which was designed to determine HLA-C alleles via polymerase chain reaction (PCR) amplification using a

| KIR haplotypes | HLA-C genotypes | Control n % | Endometriosis n % | OR 95% CI | P value |
|---------------|-----------------|-------------|-------------------|----------|---------|
| Cen-A/A       | C1/C1           | 60 68.2     | 24 79.3           | 1.786    | 0.9397 to 3.393 | 0.1016 |
| Cen-A/A       | C1/C2           | 16 59.3     | 24 68.6           | 1.5      | 0.5257 to 4.280 | 0.5932 |
| Cen-A/A       | C2/C2           | 0 0.0       | 1 100.0           | 15       | 0.1818 to 1238 | 0.3333 |
| Cen-A/B       | C1/C1           | 26 29.5     | 22 19.8           | 0.5895   | 0.3065 to 1.134 | 0.1338 |
| Cen-A/B       | C1/C2           | 10 37.0     | 11 31.4           | 0.7792   | 0.2704 to 2.245 | 0.7876 |
| Cen-A/B       | C2/C2           | 2 100.0     | 0 0.0             | 0.0667   | 0.000801 to 5.5 | 0.3333 |
| Cen-B/B       | C1/C1           | 2 2.3       | 1 0.9             | 0.9390   | 0.03484 to 4.385 | 0.5847 |
| Cen-B/B       | C1/C2           | 1 3.7       | 0 0.0             | 0.2488   | 0.009738 to 6.358 | 0.4355 |
| Cen-B/B       | C2/C2           | 0 0.0       | 0 0.0             | —        | —       | —       |
| Tel-A/A       | C1/C1           | 50 56.8     | 74 66.7           | 1.52     | 0.8529 to 2.709 | 0.1853 |
| Tel-A/A       | C1/C2           | 21 77.8     | 21 60.0           | 0.4286   | 0.1382 to 1.329 | 0.1758 |
| Tel-A/A       | C2/C2           | 0 0.0       | 1 100.0           | 15       | 0.1818 to 1238 | 0.3333 |
| Tel-A/B       | C1/C1           | 3 3.4       | 4 3.6             | 1.059    | 0.2307 to 4.863 | 0.1 |
| Tel-A/B       | C1/C2           | 0 0.0       | 0 0.0             | —        | —       | —       |
| Tel-B/B       | C1/C1           | 3 3.4       | 4 3.6             | 1.059    | 0.2307 to 4.863 | 0.1 |
| Tel-B/B       | C1/C2           | 0 0.0       | 0 0.0             | —        | —       | —       |
| Tel-B/B       | C2/C2           | 1 50.0      | 0 0.0             | 0.3333   | 0.0066 to 16.82 | 1 |

Table 7. Distribution of molecular interactions of KIR haplotypes–HLA-ligands in endometriosis and control groups. The molecular interactions of KIR haplotypes–HLA-ligands were shown in the frequency of KIR haplotypes of HLA-ligands, which was calculated from the KIR haplotypes frequency of the total number of HLA ligands. The total number of HLA ligands is shown in Table 3. Two-sided Fisher's exact test was used to estimate the differences between endometriosis and control groups. n: number of cases with relevant genotypes, OR: odds ratio, CI: confidence interval.
sequence-based typing method. Sequence data were processed using allele-typing software (AccuType™) to identify the HLA-C alleles. The genotypes of the KIR genes were analyzed using the Lifecodes KIR-sequence-specific oligonucleotide (SSO) typing kit (Immucor Transplant Diagnostics, Inc., Stamford, USA) to identify the KIR loci amplified in the sample. The presence or absence of the 16 KIR genes was determined using 20 different oligonucleotide probes targeting known KIR genes (KIR3DL3 as positive control, KIR2DL1, KIR2DL2*001-3/5, KIR2DL2*004, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DP1, KIR3DL1, KIR3DL2, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4*whole exon 4, KIR2DS4*whole exon 5, KIR2DS4*deleted exon 5, KIR2DS5, KIR3DS1, KIR3DS1*049N, and KIR3DP1). The amplicons were analyzed on a Lumines instrument according to the manufacturer’s instructions. The characteristics of full-length and truncated forms of KIR2DS4 were determined using the following three probes; probe 45: KIR2DS4*

### Statistical analyses.

HLA-C allele frequencies, the genotypes of the KIR genes and KIR–HLA-C pair frequency in endometriosis patients and control women were compared using the Fisher’s exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the GraphPad Prism software (California, USA). P value <0.05 was considered statistically significant. Multiple tests were analyzed by the Bonferroni correction using the GraphPad Prism software. The normality was analyzed by the Kolmogorov–Smirnov test using IBM SPSS statistics version 22 (New York, USA). The continuous variables of patient demographic results were analyzed by the Mann–Whitney test using the GraphPad Prism software. The discontinuous variable of dysmenorrhea was analyzed by χ² test using the GraphPad Prism software. The statistical power was analyzed by the G*Power version 3.1.9.47. The χ² value was calculated by Hardy–Weinberg analysis (χ² > 3.841 showed the subgroup was deviating from the Hardy–Weinberg equilibrium).

Received: 26 September 2019; Accepted: 24 February 2020;
Published online: 17 March 2020

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**Acknowledgements**

This work was supported by the Ministry of Science and Technology (grant number 104-2314-B-038-006, grant number 107-2314-B-038-006) (YCC). This work was financially supported by the Center for Intelligent Drug Systems and Smart Bio-devices (IDS2B) from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

**Author contributions**

Y.-C.C. designed the study, performed experiments, analysed the data and wrote the manuscripts, C.-H.C., M.-J.C., C.-W.C., P.-H.C, M.-H.Y., Y.-J.C., E.-M.T., P.-S.Y. and S.-Y.L. enrolled patients, and C.-R.T. guided the experimental design, enrolled patients and wrote the manuscripts.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Correspondence** and requests for materials should be addressed to C.-R.T.

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