荷爾蒙接受體、腫瘤抑制基因及血管生成因子相關之基因多型體與子宮內膜異位症、子宮肌瘤及泌乳素過高之關聯性

摘　要

背景與主題

子宮內膜異位症，子宮肌瘤，及泌乳素過高為婦女常見之婦科疾病，這些疾病可能是多重基因，致病因子，遺傳缺損與環境交互作用所形成，這些疾病屬於雌激素與黃體素相關之疾病；雌激素/雌激素接受體(estrogen or estrogen receptor, ER)與雄性素接受體 androgen receptor (AR)與子宮內膜異位症與子宮肌瘤之形成有關，Cytochrome P450c17 (CYP17)酵素與雌激素之代謝有關，其他賀爾蒙基因，如黃體素接受體基因，亦可能與這些疾病之致病機轉有關‧p53 基因突變可能導致細胞週期不穩定與多種腫瘤之病理機轉有關；p21 可影響 p53 基因對細胞表現之壓抑與細胞凋亡之過程有關。 基因多型體 [Single nucleotide polymorphisms (SNPs)]屬於基因體序列中最常見之 DNA 變異，本研究中針對子宮內膜異位症，子宮肌瘤，及泌乳素過高這三種常見婦科疾病，我們將偵測多種基因多型體分佈與這些疾病發生之關連性， 我們將進一步偵測多種突變型基因合併與疾病發生率，疾病嚴重度所造成之累積與加成效益， 我們亦將進一步完成這些突變型基因組合對於這些疾病發生之協同作用，交互作用與累積效益之評估。

材料與方法

所有病患區分為：(1) 嚴重子宮內膜異位症; (2) 子宮肌瘤; (3) 泌乳素過高; (4)控制組。 SNPs 包括三大組：(A)賀爾蒙/賀爾蒙接受體相關基因多型體; (B)腫瘤抑制基因相關基因多型體; (C)血管形成相關基因多型體;。 DNA 取自病患周圍血管白血球細胞，應用 polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)與 gene sequencing 來偵測 SNP 之基因變異。賀爾蒙/賀爾蒙接受體相關基因多型體包括：(1)雌性素接受體(ER*TA repeat, ER -351 A/G XbaI, ER -397 T/C PvuII); (2) CYP17 A1/A2; (3) 雄性素接受體(androgen receptor, AR, CAG repeat); (4)黃體素接受體(progesterone receptor, PR, PROGINS)。 ER dinucleotide (thymine-adenine, TA) repeat 位於 ER 基因之上游序列，根據 TA repeat 結果將 ER 基因多型體區分為'A'至'T'之 genotype，PROGINS 基因多型體為一 306-bp 之 Alu DNA 片斷 PROGINS 嵌入 PR 基因之 intron G。 腫瘤抑制基因相關基因多型體包括：(1) p53codon 11, 72, 248, p53 promoter;; (2) p21 codon 31。 Gene sequencing 之技術應用於偵測 p53 promoter 位置之基因變異。 血管形成/生長激素相關基因多型體包括：ACE A2350G, ACE A-240T, ACE intron 16 I/D。 進一步比較疾病嚴重度與各別基因突變之關聯性，疾病嚴重度分類包含子宮內膜異位之等級(stage III，IV)，子宮肌瘤大小(<，>5 ㎝)，血清泌乳素濃度(PRL<，>50 pg/ml)。 我們評估合併基因變異與疾病嚴重度之關連性，各組病患其野生/突變型 SNP 基因之比例及合併基因變異與疾病發生之關聯性予以進一步偵測與比較。

研究結果

ER*E $(TA)_{14}$, I $(TA)_{18}$ 及 O $(TA)_{24}$ 三種 genotypes 與子宮內膜異位症之高好發率有關，ER 突變之 genotypes/alleles(ER -351 G，ER -397 C) 與子宮內膜異位症及子宮肌瘤之高好發率有關，AR*M $(CAG)_{21}$ 及 AR*S $(CAG)_2$ 二種 genotype 分別與子宮內膜異位症及子宮肌瘤之高好發率有關；CYP17*A2 之 genotype 與子宮內膜異位症之高好發率有關，但與子宮肌瘤之好發率無關；PR T1/T2 之 genotype 與 allele 頻率與子宮內膜異位症及子宮肌瘤之好發率無關，但 PR*T2 相關之 genotype 與 allele 頻率與泌乳素過高之高好發率有關。 P53 codon 72*Pro genotype/allele 與子宮內膜異位症之高好發率有關，但與子宮肌瘤之好發率無關；p53 codon 11 及 248 與 p21 codon 31 於各疾病組與控制組間之表現並無差異；p53 promoter 位置共偵測出 15 處之基因變異，其中共 4 處變異頻率達到形成基因多形體 (-250 A/G, -216 T/C, -103 A/G and -33 A/G)。 這 4 組 SNP 表現於子宮肌瘤與正常控制組之分佈分別為 6.9/5.0/5.9/3.8%及 3.8/1.8/2.3/4.0%，其中-216*C 及 -103*G 與子宮肌瘤之高好發率有關。 我們發現大部分之血管形成相關基因多型體於各組間之表現具有差異，包括 ACE A2350G, ACE A-240T 及 ACE I/D，ACE insertion 相關之 genotype/alleles 與子宮內膜異位症及子宮肌瘤之高發生率有關；ACE 2350*G 及 ACE -240*T 與子宮內膜異位症之高發生率有關。

我們發現基因合併突變與疾病之較高嚴重度具有相當之關連性，其原始型/突變型基因分布比例如下：子宮內膜異位症，子宮肌瘤與泌乳素過高之原始型/突變型基因分別為：80.1/19.9%(中度子宮內膜異位症), 66.8/33.2%(重度子宮內膜異位症), 76/24% (子宮肌瘤<5 cm), 64.1/35.9% (子宮肌瘤>5 cm), 77.6/22.4% (PRL<50 pg/ml)及 57.1/42.9% (PRL>50 pg/ml)。 有關多種突變型 SNP 基因合併對於疾病發生率之影響，我們發現突變型 SNP 基因合併數量會影響疾病之發生率，在子宮內膜異位症/子宮肌瘤/泌乳素過高/正常控制組中，其突變型 SNP 基因數量之分布比例分別為：5/3/3/2% (0 組突變), 5/1/2/3% (1 組突變), 13/13/53/20% (2 組突變), 25/32/36/34% (3 組突變), 30/27/6/29% (4 組突變), 13/18/0/12% (5 組突變), 9/6/0/0% (6 組突變)。

結　論

ACE*insertion 相關 SNP 與子宮內膜異位症及子宮肌瘤之發生率有關，ER*$(TA)_{14}$, ER $(TA)_{18}$, ER$(TA)_{24,}$ AR*$(CAG)_{21,}$ CYP17*A2, P53 codon 72*Pro, ACE 2350*G 及 ACE -240*T 相關 SNP 與子宮內膜異位症之高發生率有關，AR*$(CAG)_{27}$ 相關之 SNP 與子宮肌瘤之發生率有關；p53 promoter 基因序列的一些基因變異，其中 p53 promoter -216*C 及-103*G 與與子宮肌瘤之高發生率有關；PR*T2，ACE insertion 相關 genotype/allele 與泌乳素過高有關。合併突變型 SNP 基因與疾病之較高嚴重度具有相當之關連性，有關疾病之發生率方面，我們亦發現這些突變型 SNP 基因合併發生亦與疾病之較高發生率有關，我們亦發現突變型 SNP 基因數量與這些疾病發生之趨勢有關。多種突變型 SNP 基因合併與疾病發生率，疾病嚴重度有相當關連性，患有這些疾病之病患亦可能併有多種賀爾蒙/賀爾蒙接受體相關基因，腫瘤抑制基因，及血管形成突變型 SNP 之基因組合，這些基因變異組合對於這些腫瘤細胞形成過程之引導演進與賀爾蒙分泌之變化具有一定程度之決定性影響。這些具疾病相關性之基因多型體將可能成為有效預測這些疾病發生率之有效工具。

關鍵詞：子宮內膜異位症，賀爾蒙，泌乳素過高，子宮肌瘤，基因多型體，腫瘤抑制基因

Genetic variations for hormone receptors, tumor suppressor and vasculation-related factors: correlations with endometriosis, leiomyoma and hyperprolactinemia

Summary

Background and subject

Endometriosis, leiomyoma, and hyperprolactinemia are all common gynecological diseases of pre-menopausal women. All these gynecological diseases are polygenic and multifactorial diseases. These disorders are all estrogen or progesterone-dependent diseases. Estrogen or estrogen receptor (ER) and androgen receptor (AR) play roles in the pathogenesis of endometriosis and leiomyoma. Cytochrome P450c17 ˍ CYP17) enzyme is involved with estrogen biosynthesis. Some hormone-related genes, such as progesterone receptor (PR) gene, might be involved in their pathogeneses. Mutated p53 gene is related with the instability of cell cycle progression and numerous tumorgenesis. p21 acts as a mediator of the growth suppressing and apoptosis promoting functions of p53. Single nucleotide polymorphisms (SNPs) are the most abundant types of DNA sequence variation in the human genome. Hereine we aimed to investigate the SNP distribution in three common gynecological diseases, including endometriosis, leiomyoma and hyperprolactinemia. We will detect the cumulative effects of genetic risk factors upon these disorder susceptibilities, illness severities. The related synergic, interactive and cumulative effects of these mutant genetic variations upon the illness development were assessed.

Materials and Methods

Women were divided into: (1) severe endometriosis; (2) leiomyoma; (3) hyperprolacinemia; (4) controls. These SNPs included 3 major groups, including (A) hormone/hormone receptor-related gene polymorphisms; (B) tumor suppressor gene polymorphisms; (C) vascular -related gene polymorphisms. Genomic DNA was obtained from peripheral leukocyte of subjects. The variations of DNA fragments were detected by restriction fragment length polymorphism (RFLP) or DNA sequencing. The hormone/hormone receptor-related gene polymorphisms included: (1) ER*TA repeat, ER -351 A/G XbaI, ER -397 T/C PvuII; (2) CYP17 A1/A2; (3) AR CAG repeat; (4) PR (PROGINS). The ER dinucleotide (thymine-adenine, TA) repeat polymorphism located the upstream of ER gene. The ER genotypes were classified into 'A' through 'T' (TA repeats:10 to 29). PROGINS is composed a Alu (306-bp DNA) insertion in intron G of PR gene. The tumor suppressor gene polymorphisms included: (1) p53codon 11, 72, 248, p53 promoter; (2) p21 codon 31. Sequence alignment was used to identify sequence variations in p53 promoter regions. The vascular/growth factor-related gene polymorphisms included: ACE A2350G, ACE A-240T, ACE intron 16 I/D. Illness severities were divided, including illness stages (stage III, IV endometriosis), tumor sizes (myoma<, >5 cm), and hormone levels (PRL<, >50 pg/ml). We assessed the association of combined mutant genetic variations with different degree of illness severities for individual diseases. The combined percentages of wild/mutant SNPs between each group were compared. We further assess the association of cumulative effects of combinant mutant SNPs upon increased susceptibilities for individual illnesses. The distributions for combined mutant SNPs between each group were detected.

Results

ER*E $(TA)_{14}$, I $(TA)_{18}$ and O $(TA)_{24}$ genotypes are related with higher risk of endometriosis. Higher percentages of ER mutant genotypes/alleles (-351 G, -397 C) presented in the endometriosis/leiomyoma population compared to controls. AR*M $(CAG)_{21}$ and AR*S $(CAG)_{27}$ genotypes are associated with higher susceptibility of endometriosis and leiomyoma, respectively. The CYP17*A2 was associated with higher risk of endometriosis, but not leiomyoma. PR T1/T2 genotypes and allele frequencies between endometriosis, leiomyoma and controls were non-significantly different. Higher percentage of PR*T2-related genotype and allele were noted in hyperprolactinemic women compared to other three groups. P53 codon 72*Pro related genotype/allele were associated with higher risk of endometriosis, but not leiomyoma. Distributions of p53 codon 11 and 248 and p21 codon 31 polymorphisms in each groups were non-significantly different. A total of 15 sequence variations within p53 promoter region were identified. Among these variations, 4 SNPs (-250 A/G, -216 T/C, -103 A/G, -33 A/G) were established. Allele frequencies of -250*G/-216*C/-103*G/-33*G in eiomyoma group and control group 6.9/5.0/5.9/3.8% and 3.8/1.8/2.3/4.0%. Two of them (-216*C, -103*G) are associated with higher leiomyoma susceptibility. We observed the distributions of most growth factor/vascular-related SNPs in each group were different, including ACE A2350G, ACE A-240T, and ACE I/D. ACE* insertion-related genotype and alleles were associated higher susceptibility of endometriosis and leiomyoma. ACE 2350*G, and ACE -240*T are associated higher susceptibility of endometriosis.

We observed the association of combined mutant genetic variations with higher degree of illness severities for individual diseases. In endometriosis, leiomyoma, and hyperprolactinemia cases, the combined percentages of wild/mutant SNPs were 80.1/19.9% (stage III endometriosis), 66.8/33.2% (stage IV endometriosis), 76/24% (myoma<5 cm), 64.1/35.9% (myoma>5 cm), 77.6/22.4% (PRL<50 pg/ml) and 57.1/42.9% (PRL>50 pg/ml), respectively. We observed the cumulative effects of combinant mutant SNPs upon increased illness susceptibilities. In endometriosis/leiomyoma/hyperprolactinemia/controls group, the distributions for combined mutant SNPs were listed as following: 5/3/3/2% (0 mutant), 5/1/2/3% (1 mutant), 13/13/53/20% (2 mutant), 25/32/36/34% (3 mutant), 30/27/6/29% (4 mutant), 13/18/0/12% (5 mutant), and 9/6/0/0% (6 mutant), respectively.

Conclusions

ER -351*G, ER -397*C, and ACE* insertion-genotypes/alleles are associated with higher risk of both endometriosis and leiomyoma. ER*$(TA)_{14, 18, 24,}$ AR*$(CAG)_{21,}$ CYP17*A2, P53 codon 72*Pro, ACE 2350*G, ACE -240*T and GSTM1*null related genotypes/alleles are related with higher risk of endometriosis. AR*$(CAG)_{27}$ genotypes/alleles are associated with higher susceptibility of leiomyoma. Some sequence variations were observed within p53 promoter region. P53 promoter -216*C and -103*G are associated with leiomyoma development. Higher percentage of PR*T2 and ACE*insertion-related genotype and allele were noted in hyperprolactinemic women. We observed an association of combined mutant genetic variations with higher degree of illness severities. We observed the cumulative effects of combinant mutant SNPs upon increased illness susceptibilities. We observed the cumulative effects of mutant genetic factors upon disorder susceptibilies and severities. It suggests a crucial contribution for these mutations upon the induction or progression of these tumors or hormone changes. These associated polymorphisms might become useful markers for predicting their susceptibility.

Keywords: endometriosis, hormone, hyperprolactinemia, leiomyoma, polymorphism, tumor suppressor gene.

誌　謝

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符 號 說 明

ACE	：Angiotensin I-converting enzyme
AR	：Androgen receptor
CYP17	：Cytochrome P450c17
ER	：Estrogen receptor
SNP	：Single nucleotide polymorphism