รายงานวิจัยฉบับสมบูรณ์

โครงการ

อณูระบาดวิทยาของลักษณะพันธุกรรมต่อการเกิดมะเร็งปากมดลูก ในประชากรภาคตะวันออกเฉียงเหนือของไทย

Molecular epidemiology of genetic susceptibility to cervical cancer

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มหาวิทยาลัยขอนแก่น

ธันวาคม 2552

สัญญาเลขที่ RMU5080014

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กิตติกรรมประกาศ

โครงการวิจัยเรื่อง อณูระบาดวิทยาของลักษณะพันธุกรรมต่อการเกิดมะเร็งปากมดลูกในประชากรภาคตะวันออกเฉียงเหนือของไทย ได้รับจากการสนับสนุนของสำนักกองทุนสนับสนุนการวิจัย และสำนักงานคณะกรรมการอุดมศึกษา ประจำปี 2550 จึงขอขอบพระคุณเป็นอย่างสูงมา ณ โอกาสนี้ด้วย

คณะผู้วิจัย

ธันวาคม 2552

คำนำ

โครงการวิจัยเรื่อง อณูระบาดวิทยาของลักษณะพันธุกรรมต่อการเกิดมะเร็งปากมดลูกในประชากรภาคตะวันออกเฉียงเหนือของไทย เป็นการศึกษาที่มีเป้าหมายในการหาแนวทางในการลดอุบัติการณ์การเกิดมะเร็งปากมดลูก โดยการสืบค้นสาเหตุหรือปัจจัยเสี่ยงที่จะนำไปสู่การเกิดมะเร็งชนิดนี้ของสตรีที่อาศัยอยู่ในภาคตะวันออกเฉียงเหนือ คณะผู้วิจัยหวังเป็นอย่างยิ่งว่าข้อมูลด้านอณูระบาดวิทยาจากผลการวิจัยนี้จะเป็นประโยชน์ อันจะนำไปสู่การวางแผน ป้องกัน และให้คำแนะนำที่เหมาะสมต่อไป

การวิจัยนี้สามารถดำเนินการได้ตามเป้าหมายจากการสนับสนุนของสำนักกองทุนสนับสนุนการวิจัยและสำนักงานคณะกรรมการอุดมศึกษา คณะผู้วิจัย ขอขอบพระคุณเป็นอย่างสูงมา ณ โอกาสนี้ด้วย

คณะผู้วิจัย

ธันวาคม 2552

สารบัญ

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เรื่องงานวิจัย อณูระบาดวิทยาของลักษณะพันธุกรรมต่อการเกิดมะเร็งปากมดลูกในประชากรภาคตะวันออกเฉียงเหนือของไทย (Molecular epidemiology of genetic susceptibility to cervical cancer)

รหัสโครงการ เลขที่ RMU5080014

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บทคัดย่อ

มะเร็งปากมดลูกเป็นปัญหาที่สำคัญของประเทศไทย โดยมีไวรัสฮิวแมนแปปิโลมา (HPV) ชนิดความเสี่ยงสูงเป็นสาเหตุหลักของการเกิดโรค อย่างไรก็ตามผู้ที่ติดเชื้อ HPV ไม่ได้ทำให้เกิดมะเร็งมดลูกทุกราย แสดงให้เห็นว่าอาจจะมีปัจจัยเสี่ยงอื่นๆร่วมในการเกิดมะเร็งปากมดลูก การสืบค้นสาเหตุของการเกิดมะเร็งปากมดลูกจึงเป็นหนทางหนึ่งที่สามารถจะช่วยลดอุบัติการณ์การเกิดมะเร็งชนิดนี้ ดังนั้นผู้วิจัยจึงได้ทำการศึกษาความสัมพันธ์ของปัจจัยเสี่ยงต่างๆได้แก่ ปัจจัยทางด้านพฤติกรรม ปัจจัยทางด้านพันธุกรรม รวมทั้งการติดเชื้อ HPV กับการเกิดมะเร็งปากมดลูกในสตรีภาคตะวันออกเฉียงเหนือที่มีอายุระหว่าง 27-74 ปี ในกลุ่มผู้ป่วยมะเร็งปากมดลูก(SCCA) และกลุ่มควบคุมที่เป็นอาสาสมัครที่มีสุภาพดี โดยการจัดกลุ่มผู้ป่วยมะเร็งปากมดลูกและกลุ่มควบคุมที่มีช่วงอายุกลุ่มละ 5 ปี

จากการศึกษาในกลุ่มผู้ป่วยมะเร็งปากมดลูกจำนวน 90 คน และกลุ่มอาสาสมัครที่มีสุภาพดีจำนวน 100 คน พบว่าอุบัติการณ์การติดเชื้อไวรัสฮิวแมนแปปิโลมา (HPV) ชนิดความเสี่ยงสูงในกลุ่มควบคุมและกลุ่มผู้ป่วยมะเร็งปากมดลูกมีค่าเท่ากับ 13.0% และ 86.7% ตามลำดับ พบว่าการติดเชื้อ HPV ทำให้มีโอกาสเสี่ยงต่อการเกิดมะเร็งปากมดลูกเพิ่มขึ้น 43.5 เท่า (ช่วงความเชื่อมั่น 95 % เท่ากับ 17.5-110.6; p<0.00001) ในจำนวนสตรีที่ติดเชื้อ HPV 78 คน (86.7%) จะมีสตรีที่ติดเชื้อ HPV ชนิด 16 (HPV-16) มากที่สุด คิดเป็น 70.5% รองลงมาเป็นการติดเชื้อ HPV ชนิด 18 (HPV-18) คิดเป็น 23.1% ทั้งนี้ไม่พบความแตกต่างของชนิด HPV ในกลุ่มควบคุมและกลุ่มผู้ป่วยมะเร็งปากมดลูกอย่างมีนัยสำคัญทางสถิติ รวมทั้งไม่พบความสัมพันธ์ของการติดเชื้อ HPV กับลักษณะทางพันธุกรรมของจีน *p53* codon 72 ระหว่างอัลลี proline และ arginine รวมทั้งไม่พบความแตกต่างอย่างมีนัยสำคัญของทางพันธุกรรมของจีน *p53* codon 72 กับการเกิดมะเร็งปากมดลูก ในประชากรที่ทำการศึกษา (p>0.05)

จากการศึกษาความสัมพันธ์ของปัจจัยด้านพฤติกรรมเสี่ยงกับการเกิดมะเร็งปากมดลูก พบว่าปัจจัยด้านจำนวนคู่นอน (p<0.003) อายุที่มีเพศสัมพันธ์ครั้งแรก (p<0.03) และจำนวนครั้งของการตั้งครรภ์ (p<0.006) มีความแตกต่างอย่างมีนัยสำคัญระหว่างกลุ่มควบคุมและกลุ่มผู้ป่วยมะเร็งปาก นอกจากนี้ยังพบว่าการสูบบุหรี่ของสามียังเป็นปัจจัยเสี่ยงที่สำคัญต่อการเกิดมะเร็งปากมดลูกด้วย สตรีที่มีสามีกำลังสูบบุหรี่หรือมีสามีที่เคยสูบบุหรี่จะมีความเสี่ยงต่อการเกิดมะเร็งปากมดลูกเพิ่มขึ้น 3.31 เท่า (p<0.003) หรือ 3.36 เท่า (p<0.003) ตามลำดับ

ในบุหรี่มีสารก่อมะเร็งหลายชนิดที่สามารถตรวจพบได้ที่มูกปากมดลูก สารก่อมะเร็งเหล่านี้มีผลทำลายดีเอ็นเอที่เซลล์ของปากมดลูกอันอาจนำไปสู่การซ่อมแซมเซลล์ที่ผิดปกติและกลายเป็นเซลล์มะเร็งในที่สุด อย่างไรก็ตามการได้รับสูบบุหรี่ไม่ได้ทำให้มีการพัฒนาเป็นเซลล์มะเร็งทุกกรณี แสดงว่าปัจจัยภายในร่างกายน่าจะมีส่วนสำคัญต่อการแบ่งเซลล์ที่ผิดปกติจนกลายเป็นมะเร็ง ในร่างกายมีกลไกที่จะทำลายสารพิษรวมที่กลไกการซ่อมแซมดีเอ็นเอ ที่แตกต่างกันในแต่ละบุคคล เอนซัยม์ที่เกี่ยวข้องกับกระบวนการเมแทบอลิสม (metabolizing enzymes) เป็นกลไกหนึ่งในการกำจัดสารพิษในร่างกาย โดยเฉพาะglutathione S-transferase (GST) ซึ่งเป็นเอนซัยม์กำจัดสารพิษในร่างกายมนุษย์ระยะที่ 1

(human phase I detoxification enzymes) ที่มีหลายชนิดและมีลักษณะทางพันธุกรรมที่แตกต่างกัน ดังนั้น ผู้วิจัยจึงทำการศึกษาความสัมพันธ์ของลักษณะทางพันธุกรรมของ *GSTs* ชนิด *GSTM1* และ *GSTT1* กับการเกิดมะเร็งปากมดลูก ในกลุ่มผู้ป่วยมะเร็งปากมดลูกจำนวน 90 คน และกลุ่มอาสาสมัครที่มีสุภาพดีจำนวน 94 คน

จากการศึกษาพบลักษณะจีโนไทป์แบบ *GSTM1*-null ในกลุ่มควบคุม และกลุ่มผู้ป่วยมะเร็งปากมดลูกคิดเป็น 60.0% และ 59.6% ตามลำดับ ในขณะที่ลักษณะจีโนไทป์แบบ *GSTT1*-null ในกลุ่มควบคุมและกลุ่มผู้ป่วยมะเร็งปากมดลูกคิดเป็น 40.4% และ 46.7% ตามลำดับ โดยพบว่าลักษณะจีโนไทป์แบบ *GST*-null ไม่เป็นปัจจัยเสี่ยงเสี่ยงต่อการเกิดมะเร็งปากมดลูก (p>0.05) อย่างไรก็ตาม พบว่าปัจจัยร่วมกันระหว่างลักษณะจีโนไทป์แบบ *GSTM1*-null กับ *GSTT1*-null มีแนวโน้มที่จะเพิ่มโอกาสเสี่ยงต่อการเกิดมะเร็งปากมดลูก 2.7 เท่า (95%CI=0.8-9.0, p=0.10)

นอกจากนี้ผู้วิจัยยังได้ทำการศึกษาลักษณะทางพันธุกรรมของจีนที่เกี่ยวข้องกับการซ่อมแซมดีเอ็นเอ (DNA repair genes) ต่อการเกิดมะเร็งปากมดลูกโดยเลือกศึกษาลักษณะทางพันธุกรรมของจีน XRCC1 จำนวน 2 ชนิด ได้แก่ Arg399Gln และ Arg194Trp ที่เกี่ยวข้องกับการซ่อมแซม BER และจีน XRCC3 (Thr241Met) ที่เกี่ยวข้องกับการซ่อมแซม DBS ในกลุ่มผู้ป่วยมะเร็งปากมดลูกจำนวน 111 คน และกลุ่มอาสาสมัครที่มีสุภาพดีจำนวน 118 คน ผลการศึกษาพบว่าลักษณะจีโนไทป์ของจีน *XRCC1* 194 แบบ Trp/Trp จะเพิ่มความเสี่ยงต่อการเกิดมะเร็งปากมดลูก 5.52 เท่า อย่างมีนัยสำคัญ (95%CI=1.14-26.64; p=0.03) เมื่อศึกษาในกลุ่มที่ไม่พบการติดเชื้อ HPV พบว่า ลักษณะจีโนไทป์ของจีน *XRCC1* 399 แบบ 399Arg/Gln (adjusted OR=3.69; 95%CI=1.04-13.06; p=0.04) และลักษณะจีโนไทป์ของจีน *XRCC1* 194 แบบ Arg/Trp (adjusted OR=4.13; 95%CI=1.13-15.12; p=0.03) จะเพิ่มความเสี่ยงต่อการเกิดมะเร็งปากมดลูกอย่างมีนัยสำคัญ เช่นกัน

จากผลการศึกษาแสดงว่าการติดเชื้อ HPV โดยเฉพาะ HPV-16 รวมทั้งพฤติกรรมทางเพศและการได้รับบุหรี่ทางอ้อม เป็นปัจจัยเสี่ยงที่สำคัญต่อการเกิดมะเร็งปากมดลูกของสตรีในภาคตะวันออกเฉียงเหนือ ลักษณะทางพันธุกรรมของจีน GSTM1 and GSTT1 ไม่น่าจะเกี่ยวข้องกับการเพิ่มความเสี่ยงของเกิดมะเร็งปากมดลูกอย่างมีนัยสำคัญในสตรีที่ได้รับบุหรี่ อย่างไรก็ตามลักษณะที่แตกต่างกัน ของลักษณะทางพันธุกรรมของจีนที่เกี่ยวข้องกับการซ่อมแซมดีเอ็นเอ อาจจะมีความสัมพันธ์กับการเพิ่มความเสี่ยงของการเกิดมะเร็งปากมดลูก ทั้งนี้น่าจะมีหลายปัจจัยเข้ามาเกี่ยวข้องในการพัฒนาเซลล์มดลูกที่ผิดปกติและนำไปสู่การเกิดมะเร็งปากมดลูกเมื่อมีการติดเชื้อ HPV ด้วย

Abstract

Cervical cancer is still a serious national health problem in Thailand. High risk HPV infection, a major risk for the cancer, other risk factors than should be identified to reduce the new cases of the cancer. Relationships between cervical cancer and risk factors: behaviors, genetic as well as HPV infection were investigated in the women aged 27-74 years, patients with squamous cell cervical cancer (SCCA) and healthy controls without cervical abnormalities in Northeastern Thailand. The controls and cases were matched within 5-year age group.

Among 90 patients with squamous cell cervical cancer and 100 healthy controls, prevalence of high-risk group of HPV infection in the controls and the SCCA patients were 13.0% and 86.7%, respectively. The HPV infection significantly increased the risk for cervical cancer 43.5 -fold (95 % confidential interval: 17.5-110.6; $p<0.00001$). Among HPV carrier patients with SCCA (n=78), HPV-16 was also prominent (70.5%) followed by HPV-18 (23.1%). There was no statistical difference in the subtype distribution between the SCCA and the control groups. The relationships between HPV infection and *p53* codon 72 polymorphism, proline and arginine allele was studied. There was no significant association between genotype distribution of the *p53* codon 72 polymorphism and the HPV infection. In addition, there was no significant difference in allele and genotype distribution between the SCCA and the control groups ($p>0.05$).

Relationships between cervical cancer and behavioral risk factor were studied. Significant difference was observed in the number of sexual partners ($p<0.003$), age at the first sexual intercourse ($p<0.03$) and number of partities ($p<0.006$). After adjusted by age and *p53* genotype significant difference was still observed in the number of sexual partners ($p<0.017$). The partners' smoking increased the risk to develop SCCA. Increased odd ratios were observed when the partner had smoking history both at present (3.31;$p<0.003$) and in the past (*3.36;*$p<0.003$). HPV infection was confirmed as a critical risk factor for the cervical cancer development while the *p53* codon 72 polymorphism itself may not be a risk factor for cervical cancer in Northeast Thailand. Since the polymorphism of the *p53* itself as well as in combination with HPV infection may not be a genetic risk for cervical cancer, much attention should be paid to other risk factors such as sexual behaviors and smoking.

Carcinogens have been detected in the cervical mucus of smokers, inhaled tobacco-derived components may damage smokers' cervical cellular DNA, not all smokers develop cervical cancer. The difference, therefore, in the metabolic efficiency of tobacco smoke pro-carcinogens is thought to be the individual's susceptibility to cervical cancer. Among the metabolizing enzymes, glutathione S-transferase (GST) is related to human phase I detoxification enzymes. Therefore, the relationships between genetic polymorphisms of the *GSTs* (*GSTM1* and *GSTT1*) and cervical cancer, the null genotype of each gene was studied in squamous cell cervical cancer (SCCA) patients (n=90) and controls (n=94).

The prevalence of the *GSTM1*-null genotype in the controls and SCCA patients was 59.6% and 60.0%, respectively, whereas those of the *GSTT1*-null genotype in the control and SCCA patients was 40.4% and 46.7%, respectively. Neither of the *GST*-null genotypes increased the risk for SCCA (p>0.05); however, the combination of the *GSTM-1* and *GSTT1*-null genotypes showed a trend to an increased risk for developing cervical cancer with adjusted OR=2.7 (95%CI=0.8-9.0, p=0.10). Genetic polymorphism of GSTM1 and GSTT1 was not a significant risk for cervical cancer in either tobacco-smokers or non-smokers. A different contribution of the *GST* genotype to cancer risk may be attributed to a different, as yet undefined, property of the enzymes.

Since the influence of the polymorphisms of DNA repair genes on the development of cervical cancer was unknown, we have selected BER related XRCC1 and DBS related XRCC3 to test the contribution of their polymorphisms, XRCC1 Arg399Gln and Arg194Trp and XRCC3 Thr241Met, to develop cervical cancer. In this study, cases (n=111) were defined as squamous cell cervical cancer and controls (n=118) were recruited The *XRCC1* 194Trp/Trp genotype significantly increased the risk for cervical cancer (OR=5.52; 95%CI=1.14-26.64; p=0.03). Among the HPV infection negative group, significantly higher risks for SCCA were visualized for *XRCC1* 399Arg/Gln (adjusted OR=3.69; 95%CI=1.04-13.06; p=0.04) and *XRCC1* 194Arg/Trp (adjusted OR=4.13; 95%CI=1.13-15.12; p=0.03).

This study indicates HPV infection is identified as a critical risk factor, particular HPV-16 for the cervical cancer development in Northeast Thailand . To other risk factors such as sexual behaviors and smoking may serve as cofactors to increase risk for cervical carcinoma in the presence of HPV. The null genotype of phase I detoxification enzymes, GSTM1 and GSTT1, did not increase the risk for SCCA in smokers. That variant types of DNA repair genes play partial roles in modifying individual susceptibility to cervical cancer. Since cervical cancer is a multi-factorial disease, the contribution of repair enzymes if it ever exists to the development of cervical caner is concealed by HPV infection.

เนื้อหางานวิจัย

เรื่อง อณูระบาดวิทยาของลักษณะพันธุกรรมต่อการเกิดมะเร็งปากมดลูกในประชากรภาคตะวันออกเฉียงเหนือของไทย (Molecular epidemiology of genetic susceptibility to cervical cancer)

บทนำ

ในปัจจุบัน มะเร็งเป็นสาเหตุสำคัญอันหนึ่งที่ทำให้ประเทศมีการสูญเสียทรัพยากรบุคคลและเศรษฐกิจอย่างมหาศาล การเกิดมะเร็งส่วนใหญ่เชื่อว่ามีสาเหตุจากปัจจัยที่ซับซ้อนหลายประการ เช่น พันธุกรรม สิ่งแวดล้อม และวิถีการดำเนินชีวิต เป็นต้น มะเร็งปากมดลูกเป็นโรคมะเร็งสำคัญชนิดหนึ่งที่พบมากในสตรีที่อาศัยอยู่ในภาคตะวันออกเฉียงเหนือของไทยกว่าทศวรรษ แม้ว่าการติดเชื้อไวรัสฮิวแมนแปปิลโลมา ((human papilloma virus, HPV) จะเป็นปัจจัยเสี่ยงที่สำคัญต่อการเกิดโรคนี้ แต่ผู้ที่เป็นมะเร็งปากมดลูกไม่ได้มีสาเหตุจากการติดเชื้อ HPV ทุกราย แสดงให้เห็นว่าน่าจะมีปัจจัยอื่นที่มีผลเกี่ยวข้องกับการเกิดมะเร็งปากมดลูก ปัจจัยเสี่ยงด้านพฤติกรรมทางเพศ และสูบบุหรี่ เช่น การมีเพศสัมพันธ์เมื่ออายุน้อยกว่า 17 ปี การมีคู่นอนมากกว่า 1 คน และการได้รับบุหรี่ทางอ้อม (passive smoking) จากคนใกล้ชิด (สามี) เป็นปัจจัยเสี่ยงสูงที่มีนัยสำคัญต่อการเกิดมะเร็งปากมดลูกในสตรี อย่างไรก็ตามแม้ว่าสตรีที่ได้รับบุหรี่ทางอ้อมจะมีโอกาสเกิดมะเร็งปากมดลูก รวมทั้งติดเชื้อ HPV ได้ง่ายกว่าสตรีที่ไม่ได้รับบุหรี่ แต่สตรีที่ได้รับบุหรี่ไม่ได้เป็นมะเร็งปากมดลูก และ/หรือติดเชื้อ HPV ทุกราย

ในควันบุหรี่มีสารก่อมะเร็งหลายชนิด เช่น polycyclic aromatic hydrocarbons, nicotine, hydrazine, nitrosamines เป็นต้น เมื่อสารก่อมะเร็งนี้เข้าสู่ร่างกายจะถูกเมแทบอไลซ์ด้วยเอ็นซัยม์ cytochrome P450 และ glutathione S-transferase (GST) ซึ่งเอ็นซัยม์เหล่านี้จะเป็นตัวแปรที่สำคัญต่อฤทธิ์ของสารก่อมะเร็งในร่างกายโดยอาจทำให้สารก่อมะเร็ง มีฤทธิ์เพิ่มมากขึ้นจนมีผลทำให้ร่างกายเกิดมะเร็งหรือทำให้สารก่อมะเร็งมีฤทธิ์ลดลงแล้วถูกขจัดออกไปจากร่างกาย สารเคมีในควันบุหรี่เหล่านี้ยังมีผลกระทบต่อการทำงานของระบบภูมิคุ้มกันของร่างกายในหลายอวัยวะ รวมทั้งลดการทำงานของกระบวนการต่อต้านเชื้อโรคในอวัยวะสืบพันธุ์ของสตรีด้วย ยังผลให้สตรีที่ได้รับบุหรี่มีโอกาสติดเชื้อและเป็นโรคในระบบสืบพันธุ์ได้ง่ายขึ้น อย่างไรก็ตาม แม้ว่าการติดเชื้อ HPV จะเป็นปัจจัยหลักของการเกิดมะเร็งปากมดลูก แต่สตรีที่มีเชื้อ HPV ไม่ได้เป็นมะเร็งปากมดลูกทุกราย ทั้งนี้อาจจะขึ้นอยู่กับการทำงานของระบบภูมิคุ้มกันของร่างกายในแต่ละบุคคลที่มีความแตกต่างกันโดยเฉพาะ lymphokines ที่ทำหน้าที่สำคัญในการป้องกัน และกำจัดเชื้อโรคที่เข้าสู่ร่างกาย รวมทั้งขึ้นอยู่กับการทำงานของกระบวนการซ่อมแซม DNA ในร่างกายเพื่อให้เซลล์ที่ได้รับบาดเจ็บจากการทำลายของเชื้อโรคดังกล่าวสามารถกลับมาทำหน้าที่ได้ตามปกติ ซึ่งจะส่งผลต่อการพัฒนาและการเกิดโรคที่แตกต่างกัน จากการพัฒนาด้านเทคโนโลยีชีวภาพที่ทันสมัยในปัจจุบัน ช่วยให้สามารถศึกษาถึงพันธุกรรมที่มีลักษณะที่แตกต่างกันในปัจเจกบุคคล ซึ่งเป็นสาเหตุสำคัญอันหนึ่งต่อการก่อโรคได้ง่ายและสะดวกขึ้น ในบรรดาจีนที่เกี่ยวข้องกับการเกิดมะเร็ง จีนที่เกี่ยวข้องกับเมแทบอลิซึมของเอ็นซัยม์ จีนที่เกี่ยวข้องกับการซ่อมแซมดีเอ็นเอ และจีนที่เกี่ยวข้องกับระบบภูมิคุ้มกันของร่างกาย เป็นจีนที่สำคัญต่อการเกิดมะเร็งหลายชนิด อย่างไรก็ตาม แม้ว่าได้มีการศึกษาความสัมพันธ์ของจีนบางส่วนกับการเกิดมะเร็งมาก่อนหน้านี้ เนื่องจากลักษณะของกรรมพันธุ์มีความแตกต่างกันในแต่ละเชื้อชาติ และยังขาดข้อมูลด้านความสัมพันธ์ของการเกิดมะเร็งกับกลุ่มจีนที่เสี่ยงต่อการเกิดมะเร็งที่แน่ชัด อีกทั้งยังไม่มีรายงานการศึกษาจีนกลุ่มนี้กับการเกิดมะเร็งปากมดลูกในประชากรของไทย ดังนั้นผลจากการศึกษาในครั้งนี้จะทำให้

ได้รู้ถึงลักษณะจีนสำคัญที่เกี่ยวข้องกับการเกิดมะเร็งปากมดลูกในประชากรของไทย ซึ่งอาจจะสามารถนำไปใช้เป็นเครื่องหมายด้านกรรมพันธุ์ ที่จะบ่งชี้ถึงเป็นสาเหตุและปัจจัยด้านพันธุกรรมที่มีผลต่อการเกิดมะเร็งปากมดลูก ซึ่งนำไปช่วยในการวินิจฉัย ป้องกัน และการรักษาที่ถูกต้องเหมาะสม สามารถลดอุบัติการณ์การเกิดมะเร็งปากมดลูก ลดการสูญเสียทรัพยากรบุคคล ลดการสูญเสียด้านเศรษฐกิจ รวมถึงลดปัญหาครอบครัว และสังคมของไทยในภาพรวม

วัตถุประสงค์

เพื่อสืบค้น high risk genetic backgrounds ตลอดจนพฤติกรรมเสี่ยงที่มีความสัมพันธ์และเกี่ยวข้องกับการติดเชื้อไวรัสและการเกิดมะเร็งปากมดลูก ซึ่งประกอบด้วย จีน P53 จีนที่เกี่ยวข้องกับเมแทบอลิซึม ได้แก่ P450 (CYP1A1, CYP2A6, CYP2E1, CYP2D6) และ GST (M1, T1), และจีนที่เกี่ยวข้องกับการซ่อมแซม DNA ได้แก่ XRCC11 XRCC16 และ XRCC3

วิธีวิจัย

1. ประชากร

ก. กลุ่มศึกษา (สตรีที่เข้ามารับการรักษาในโรงพยาบาลศรีนครินทร์และได้รับการวินิจฉัยจากแพทย์ว่าเป็นมะเร็งปากมดลูก หรืออาสาสมัครที่มีสุขภาพแข็งแรงที่ได้รับการตรวจมูกที่ปากมดลูก และได้รับการวินิจฉัยจากแพทย์ว่าปากมดลูกปกติไม่อยู่ในภาวะก่อนการเป็นมะเร็งปากมดลูก, มะเร็งปากมดลูก หรือไม่มีความผิดปกติใด) จำนวน 100 คน

ข. กลุ่มควบคุม (สตรีที่เข้ามารับการรักษาในโรงพยาบาลศรีนครินทร์และได้รับการวินิจฉัยจากแพทย์ว่าเป็นมะเร็งปากมดลูก หรืออาสาสมัครที่มีสุขภาพแข็งแรงที่ได้รับการตรวจมูกที่ปากมดลูก และได้รับการวินิจฉัยจากแพทย์ว่าปากมดลูกปกติไม่อยู่ในภาวะก่อนการเป็นมะเร็งปากมดลูก, มะเร็งปากมดลูก หรือไม่มีความผิดปกติใด) จำนวน 100 คน

2. วิธีศึกษา

ก. บันทึกข้อมูลพื้นฐานพฤติกรรมเสี่ยงด้านการสืบพันธุ์ และประวัติการสูบบุหรี่

ข. เก็บตัวอย่างเซลล์จากปากมดลูก ในสารละลาย PBS เพื่อนำมาสกัด DNA โดยใช้ kit ของ Qiangen จากประเทศเยอรมันนี ตรวจวัด HPV ด้วย kit ของ Takara ประเทศญี่ปุ่น โดยวิธี PCR แล้วตัดด้วยเอ็นซัยม์ Ava II, Ava I, Bgl II AccI และ Afa I เพื่อแยกชนิด 16, 18, 31, 33, 52 และ 58

ค. เก็บเลือด จำนวน 5 มิลลิลิตรจากหลอดเลือดดำที่แขนของประชากรทั้ง 2 กลุ่มใส่ในหลอดทดลองที่มี heparin แล้วนำมาปั่นแยกพลาสมาและเม็ดเลือดขาว เก็บไว้ที่ – 80 °C ส่วนของเม็ดเลือดจะนำมาสกัด DNA โดยวิธี NaI และศึกษาลักษณะทางพันธุกรรมของจีนพี 53, detoxification enzymes และ DNA repair โดยวิธี PCR ตรวจสอบ genotypes ของ p53 และ DNA repair โดยวิธี RFLP

3 การวิเคราะห์ข้อมูล

การประมวลผลและวิเคราะห์ข้อมูลทางสถิติด้วยคอมพิวเตอร์ใช้ χ^2 วิเคราะห์ haplotype และ genotyping คำนวณ allele frequencies ทดสอบด้วย Hardy-Weinberg's equilibrium ซึ่งจะทำการเปรียบเทียบ genotype frequencies ระหว่างกลุ่มผู้ป่วยมะเร็งที่ศึกษากับกลุ่มควบคุม ด้วยโปรแกรม STATA และ Fisher's exact test เพื่อวิเคราะห์ความสัมพันธ์ระหว่างลักษณะพันธุกรรมของจีน การติดเชื้อไวรัส และปัจจัยเสี่ยงอื่นๆ กับการเกิดมะเร็งของปากมดลูก แสดงผลเป็นค่าความเสี่ยงสัมพัทธ์คือ odds ratio และช่วงแห่งความเชื่อมั่นที่ 95% เพื่อค้นหาปัจจัยเสี่ยงต่อการเกิดมะเร็งด้วยการหาค่า Odd ratio (ORs)

ผลการทดลอง

HPV infection and Cervical cancer

Prevalence of the high-risk group of HPV infection in the control and the SCCA patients was 13% and 86.7%, respectively (Table 1). The HPV infection significantly increased the risk for cervical cancer 43.5 -fold (95 % CI:17.5-110.6; $p<0.00001$).

As for the genotype distribution, infection of HPV-16, -18, -31, -33, -35, -52b and -58 was found with a variety of frequency in the subjects (Table 3). In HPV carriers of the controls (n=13), HPV-16 was the commonest (8/13) followed by HPV-58 (3/13). Among HPV carrying patients with SCCA (n=78), HPV-16 was also prominent (70.5%) followed by HPV-18 (24.4%). There was no statistical difference in the genotype distribution between the SCCA and the control groups. Several combinations of double and triple infections were observed (Table 2). Prevalence of HPV infection and the distribution of the *p53* codon 72 genotype were tested (Table 3).

Table 1 Prevalence of infection with high-risk group of HPV infection

	HPV status		
Group	negative	positive	OR [95% CI]
Controls	87	13	
Cases	12	78	43.5 [17.49-110.64]**

OR was calculated against negative for HPV infection. **$p<0.00001$

Table 2 Distribution of HPV genotypes

HPV genotypes	Control[a] (n=100)	SCCA[b,c] (n=90)
HPV-16	8	55
HPV-18	1	19
HPV-31	2	0
HPV-33	1	1
HPV-35	0	3
HPV-52b	1	3
HPV-58	3	9
not typed	3	2

[a]double infection of -16/-52b (n=1), -16/-58 (n=1), -16/not typed(n=1), -31/-58 (n=1), -31/not typed (n=1) and -33/-58 (n=1) were observed.

[b]double infection of -16/-18 (n=2), -16/-35 (n=2), -16/-52b (n=2), -16/not typed(n=1), -33/-58 (n=1), -52b/-58(n=1) and -58/not typed (n=1) were observed.

[c]triple infection of -16/-18/58 (n=2) were observed.

Table 3 Genetic distribution of p53 codon 72 polymorphism and HPV infection

Group	HPV Infection	Genotype distribution		
		Pro/Pro	Pro/Arg	Arg/Arg
Control (n=100)	negative	23	43	21
	positive	2	10	1
Case (n=90)	negative	1	8	3
	positive	16	42	20

Behavioral risk and Cervical cancer

The Pro and Arg allele frequency and genotype distribution in SCCA and the control are shown in Table 4. The proportion of Pro/Pro, Pro/Arg and Arg/Arg genotypes in the SCCA patients was 18.9, 55.6 and 25.6 % and in the controls was 25.0, 53.0 and 22.0 %, respectively. The Arg/Arg genotype increased OR to 2.76-fold. There were no significant differences in the proportion of the p53 codon 72 in the SCCA and the control groups.

As for the patterns of sexual behaviors and life style, statistically significant difference was observed in the number of sexual partners, age at the first sexual intercourse and number of parities with p-value of 0.003, 0.03 and 0.006, respectively. After adjusted by age and p53 genotype, significant difference was still observed in the number of sexual partners (p=0.017). Adjusted OR[95%CI] for the plural sexual partner was 2.37[1.16-4.81]. Proportion of those who had plural sexual partner was higher in SCCA patients (31.4%) than in the controls (16.0%). Moreover, the partners' smoking increased the risk to develop SCCA. Increased ORs were observed when the partner had smoking history both at present (3.31;p<0.0003) and in the past (3.36;p<0.0003), adjusted OR also showed significance(3.52;p<0.007 and 10.48;p<0.002, respectively)

Table 4 p53 codon 72 allele and genotype frequencies with OR in SCCA patients and healthy controls

Groups	Allele frequencies OR [95% CI]*		Genotype distribution (%) OR [95% CI]*		
	P	A	P/P	P/A	A/A
Cases	0.47	0.53	17(18.9%)	50(55.6%)	23(25.6%)
			1.51[0.82-2.79	1.39[0.36-3.08	1.54[0.60-3.92]
			1.00[0.39-2.58]	1.34[0.52-3.49]	2.76[0.63-12.05]
Controls	0.57	0.43	25(25%)	53(53%)	22(22%)

*ORs were calculated against the P allele and P/P genotype; the upper is crude and the lower is adjusted for age, age at first intercourse, number of sexual partners, number of pregnancies and smoking.

Table 5 Selected risk factors for SCCA

Variables	Subject number (%)		Crude OR*	Adjusted OR*
	SCCA	Controls	OR [95% CI]*	
Age at menarche				
>14 years	68(75.6)	68(68.0)	1.00	1.00
≤14 years	22(24,4)	32(32.0)	0.68[.43-1.36]	.67[.29-1.52]
Number of sexual partners				
≤1	59(65.6)	84(84.0)	1.00	1.00
>1	31(31.4)	16(16.0))	2.76[1.32-5.90]**	2.37[1.16-4.81]*
Age at the first intercourse				
>17 years	56(72.2)	85(85.0)	1.00	1.00
≤17 years	25(27.8)	15(15.0)	2.18[1.01-4.81]*	1.42[0.58-3.46]
Age at the first birth				
>20 years	44(48.9)	60(60.0)	1.00	1.00
≤20 years	46(51.1)	40(40.0)	1.57[.85-2.90]	0.32[0.07-10.52]
Number of pregnancies				
≤3	46(51.1)	60(60.0)	1.00	1.00
>3	44(48.9)	40(40.0)	1.43[0.77-2.65]	1.04[0.47-2.29]
Number of abortions				
0	58(65.2)	64(64.0)	1.00	1.00
1	21(23.6)	25(25.0)	0.98[]0.44-1.93]	1.08[0.46-2.52]
>1	10(11.2)	11(11.0)	1.00[0.35-2.81]	0.90[0.30-2.72]
Number of parities				
≤3	57(63.3)	80(80.0)	1.00	1.00
>3	33(36.7)	20(20.0)	2.31[1.15-4.70]*	1.74[0.70-4.28]
Use of oral contraceptive pills				
Not used	47(52.2)	53(53.0)	1.00	1.00
1-4 years	19(21.1)	36(36.0)	0.59[0.28-1.24]	0.85[0.37-1.94]
5-9 years	12(13.5)	5(5.0)	2.70[0.80-10.46]	2.50[0.65-9.52]
≥10 years	12(13.5)	6(6.0)	2.22[0.71-7.87]	1.65[0.46-5.84]
Use of oral contraceptive injection				
No	71(78.9)	69(69.0)	1.00	1.00
Yes	19(21.1)	31(31.0)	0.85[0.43-1.67]	1.56[0.42-1.33]
Use of IUD				
No	65(72.2)	69(69.0)	1.00	1.00
Yes	25(27.8)	31(31.0)	0.59[0.29-1.21]	0.81[0.37-1.78]

History of STD				
Subjects				
No	74(82.2)	69(69.0)	1.00	1.00
Yes	16(17.8)	10(10.0)	1.95[0.77-5.08]	2.05[0.74-5.70]
Partners				
No	74(82.2)	91(91.0)	1.00	1.00
Yes	16(17.8)	9(9.0)	2.18[0.85-5.93]	2.24[0.79-6.40]
History of smoking				
Subjects				
Non-smoker	92(91.1)	91(91.0)	1.00	1.00
Present smoker	7(7.8)	9(9.0)	0.98[0.32-3.03]	0.93[0.35-2.52]
Past smoker	1(1.1)	0(0.0)		
Partners				
Non-smoker	15(16.7)	40(40.0)	1.00	1.00
Present smoker	51(56.7)	41(41.0)	3.31[1.52-7.35]**	3.52[1.40-8.85] **
Past smoker	24 (26.7)	19(19.0)	3.36[1.33-8.57]**	10.48[2.31-47.41]**
Circumcision of partner[c]				
Yes	6(8.6)	9(10.6)	1.00	1.00
No	64(91.4)	76(89.4)	1.26[0.38-4.55]	1.52[0.36-6.47]

[a]OR were presented with 95% CI in the bracket; *: $p<0.05$; **: $p<0.01$. [b]Adjusted for age and p53 genotype.

[c]Total number of SCCA and controls was 70 and 85, respectively.

Detoxification enzymes and Cervical cancer

Table 6 shows the genotype distribution of *GSTM1 and GSTT1* for the cases and controls. The prevalence of the *GSTM1*-null genotype in the control and SCCA patients was 59.6% and 60.0%, respectively, whereas that of the *GSTT1*-null genotype in the control and SCCA patients was 40.4% and 46.7%, respectively. Neither the *GSTM1*- nor *GSTT1*-null genotypes increased the risk for SCCA (p>0.05). The combination of the *GSTM1*- and *GSTT1*-null genotypes showed a trend to increasing the risk of developing cervical cancer with an adjusted OR of 2.72 (95%CI=0.82-9.03, p= 0.10). This trend was also observed when we tested smokers (Table 7); namely, the *GSTM1*- or *GSTT1*-null genotype did not increase the risk for SCCA in smokers (p>0.05), whereas an increased risk for SCCA with an adjusted OR=1.82 (95%CI=43-7.67, p=0.41) was observed in the *GST* double negative carriers.

Table 6 Genetic polymorphism of *GSTM1* and *GSTT1* in the cervical cancer

Genotypes	Subjects, n(%)		OR	Adjusted OR*
	Cases	Controls	[95%CI,p-value]	[95%CI,p-value]
GSTM1				
+	36(40.0)	38(40.4)	1	
-	54(60.0)	56(59.6)	1.02	0.66
			[0.54-1.91,0.95]	[0.30-1.48, 0.32]
GSTT1				
+	48(53.3)	56(59.6)	1	
-	42(46.7)	38(40.4)	1.29	0.72
			[0.69-2.41,0.39]	[0.29-1.80, 0.48]
GSTM1/T1				
+/+	20(22.2)	18(19.1)	1	
+/-	28(31.1)	38(40.4)	0.67	-**
			[0.35-1.78, 0.19]	
-/+	16(17.8)	20(21.3)	0.80	-**
			[0.38-1.77, 0.55]	
-/-	26(28.9)	18(19.1)	1.72	2.72
			[0.82-3.64, 0.12]	[0.82-9.03, 0.10]

OR [95% CI] = odds ratios [95% confidence interval], * Adjusted for age, *p53* genotypes, smoking and HPV status, **drop because of co-linearity

Table 7 GST genotypes and risk for SCCA among smokers

Genotypes	Subjects, n(%)		OR	Adjusted OR*
	Cases	Controls	[95%CI,p-value]	[95%CI,p-value]
GSTM1				
+	30(41.1)	24(42.1)	1	1
-	43(58.9)	33(57.9)	1.00	0.78
			[0.46-2.15, 1.00]	[0.30-2.03, 0.62]
GSTT1				
+	39(53.4)	34(59.6)	1	1
-	34(46.6)	23(40.4)	1.35	1.74
			[0.63-2.91, 0.43]	[0.68-4.45, 0.25]
GSTM1/T1				
+/+	16(21.9)	12(21.4)	1	1
+/-	23(31.5)	22(39.3)	0.71	-**
			[0.32-1.57, 0.36]	
-/+	14(19.2)	11(19.6)	0.97	-**
			[0.37-2.61, 0.95]	
-/-	20(27.4)	11(19.6)	1.54	1.82
			[0.62-3.96, 0.31]	[0.43-7.67, 0.41]

OR [95% CI] = Odds ratios [95%confidence interval], * Adjusted for age, *p53* genotypes and HPV status, **drop because of collinearity

Polymorphisms of DNA repair genes and Cervical cancer

The allele frequencies and distribution of genotypes of the *XRCC1* codon 399 and 194, and *XRCC3* codon 241 are shown in Table 8. No significant deviation from Hardy-Weinberg equilibrium in the genotype distribution for the three loci was confirmed in the controls. The prevalence of the *XRCC1* 194Trp allele (T) was not significantly different in cases and controls (p>0.05) but *XRCC1* 194Trp/Trp genotype significantly increased the risk for cervical cancer (OR=5.52; 95%CI=1.14-26.64; p=0.03), whereas heterozygous genotype did not (OR=1.18; 95%CI=0.69-2.01; p=0.54). *XRCC1* 399 and *XRCC3* 241 polymorphisms did not alter the risk for the development of cervical cancer when we analyzed by genotype and allele distribution (Table 8). When ORs were calculated for combined genotypes of XRCC1 399 and 194, there was a trend to increase the risk for the cancer in Arg/Arg-Trp/Trp (G/G-T/T) genotype (OR=4.31; 95%CI=0.82-22.53; p=0.08) (Table 9). When genotypes were combined for three loci, the trend of increased risk in the presence of XRCC1 194Trp/Trp genotype was still observed (OR=4.08; 95%CI=0.77-21.54; p=0.09) (Table 10).

Interaction between *XRCC* genotypes and the risk for SCCA by the status of HPV infection was analyzed (Table 11). Among the HPV infection negative group, significantly higher risks for SCCA were visualized for *XRCC1* 399Arg/Gln (adjusted OR=3.69; 95%CI=1.04-13.06; p=0.04) and *XRCC1* 194Arg/Trp (adjusted OR=4.13; 95%CI=1.13-15.12; p=0.03). Other genotypes with the *XRCC1* 339Glu allele or the *XRCC1* 194Trp allele consistently showed higher risks even though the p values were not less than 0.05. When risk of the XRCC polymorphisms for SCCA was evaluated by the smoking status, none of the genotypes showed deviation in the risk for the cancer statistically (Table 12).

Table 8 Risk of XRCC genotypes for SCCA

Genotype and frequency of variant allele	Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
	Cases	Controls		
XRCC1 codon 399				
Arg/Arg	66	69	1	1
Arg/Gln	41	44	0.97 [0.56-1.67], 0.95	1.45 [0.66- 3.18], 0.34
Gln/Gln	4	5	0.84 [0.21-3.25], 0.79	2.41 [0.36-16.05], 0.36
Arg/Gln+Gln/Gln	45	49	0.96 [0.57-1.63], 0.88	1.47 [0.69- 3.37], 0.31
freq. allele Gln	0.221	0.229 (p>0.05)		
XRCC1 codon 194				
Arg/Arg	53	65	1	1
Arg/Trp	49	51	1.18 [0.69- 2.01], 0.54	1.21 [0.57- 2.59], 0.61
Trp/Trp	9	2	5.52 [1.14-26.64], 0.03	6.73 [0.92-48.78], 0.06
Arg/Trp+Trp/Trp	58	53	1.34 [0.79- 2.25], 0.27	1.38 [0.67- 2.87], 0.38
freq. allele Trp	0.302	0.233 (p>0.05)		

XRCC3 codon 241				
Thr/Thr	101	106	1	1
Thr/Met	10	12	0.87 [0.36- 2.11],0.76	2.13 [0.61- 7.43], 0.23
freq. allele Met	0.045	0.051 (p>0.05)		

OR [95% CI]: Odds ratios [95% confidence interval], *adjusted with multiple logistic regression for age, HPV status and smoking

Table 9 Risk of combined two XRCC1 polymorphisms for SCCA

XRCC genotype		Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
399	194	Cases	Controls		
Arg/Arg	Arg/Arg	26	32	1	1
Arg/Arg	Arg/Trp	33	35	1.16 [0.57- 2.34], 0.68	0.81 [0.30- 2.17], 0.68
Arg/Arg	Trp/Trp	7	2	4.31 [0.82-22.53], 0.08	2.93 [0.34-25.14], 0.32
Arg/Gln	Arg/Arg	24	28	1.05 [0.49- 2.24], 0.89	0.92 [0.32- 2.63], 0.88
Gln/Gln	Arg/Arg	3	5	0.74 [0.16- 3.38], 0.69	1.20 [0.14-10.52], 0.87
Arg/Gln	Arg/Trp	16	16	1.23 [0.52- 2.92], 0.64	1.96 [0.57- 6.73], 0.28

OR [95% CI]: Odds ratios [95%confidence interval],*adjusted with multiple logistic regression for age, HPV status and smoking

Table 10 Combination of XRCCs genotypes and risk for SCCA

Genotype			Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
XRCC1		*XRCC3*				
399	194	241	Cases	Controls		
Arg/Arg	Arg/Arg	Thr/Thr	24	28	1	1
Arg/Arg	Arg/Arg	Thr/Met	2	4	0.58 [0.09- 3.47], 0.55	1.00 [0.08-11.71], 0.99
Arg/Arg	Arg/Trp	Thr/Thr	31	31	1.16 [0.56- 2.44], 0.68	0.78 [0.28- 2,20], 0.65
Arg/Arg	Arg/Trp	Thr/Met	2	4	0.58 [0.09- 3.46], 0.55	0.94 [0.07-12.83], 0.97
Arg/Arg	Trp/Trp	Thr/Thr	7	2	4.08 [0.77-21.54], 0.09	0.72 [0.31-23.74], 0.37
Arg/Gln	Arg/Arg	Thr/Thr	20	24	0.97 [0.43- 2.17], 0.94	0.82 [0.26- 2.52], 0.73
Arg/Gln	Arg/Arg	Thr/Met	4	4	1.17 [0.26- 5.17], 0.84	2.41 [0.32-18.22], 0.39
Arg/Gln	Arg/Trp	Thr/Thr	14	16	1.02 [0.41- 2.51], 0.96	1.51 [0.42- 5.48], 0.52
Gln/Gln	Arg/Ar	Thr/Thr	3	5	0.70 [0.15- 3.23], 0.65	1.10 [0.12- 9.69], 0.25

OR [95% CI]: Odds ratios [95%confidence interval],*adjusted with multiple logistic regression for age, HPV status and smoking

Table 11 XRCC genotype, HPV status and risk for SCCA

Genotype	Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
	Cases	Controls		
HPV negative				
XRCC1 codon 399				
Arg/Arg	5	56	1	1
Arg/Gln	9	35	2.88 [0.89- 9.29], 0.08	3.69 [1.04-13.06], 0.04
Gln/Gln	1	5	2.24 [0.22-23.11], 0.49	4.53 [0.34-59.67], 0.25
Arg/Gln+Gln/Gln	10	40	2.80 [0.89- 8.82], 0.08	2.83[0.89- 9.04], 0.08
XRCC1 codon 194				
Arg/Arg	5	56	1	1
Arg/Trp	9	38	2.65 [0.82- 8.53], 0.10	4.13 [1.13-15.12], 0.03
Trp/Trp	1	2	5.60 [0.43-73.08], 0.19	7.23 [0.50-103.89], 0.14
Arg/Trp+Trp/Trp	10	40	2.80 [0.89- 8.82], 0.08	3.06 [0.95- 9.83], 0.06
XRCC3 codon 241				
Thr/Thr	13	84	1	1
Thr/Met	2	12	1.07 [0.21- 5.37], 0.93	1.55 [0.27- 8.74], 0.62
HPV positive				
XRCC1 codon 399				
Arg/Arg	61	13	1	1
Arg/Gln	32	9	0.76 [0.29- 1.96], 0.57	0.71 [0.26- 1.93], 0.51
Arg/Gln+Gln/Gln	35	9	0.83 [0.32- 2.13], 0.70	0.82 [0.32- 2.14], 0.70
XRCC1 codon 194				
Arg/Arg	48	9	1	1
Arg/Trp	40	13	0.57 [0.22- 1.48], 0.25	0.57 [0.21- 1.55], 0.27
Arg/Trp+Trp/Trp	48	13	0.69 [0.27- 1.77], 0.44	0.69 [0.27- 1.78], 0.45

OR [95% CI] = Odds ratios [95%confidence interval], * adjusted with multiple logistic regression for age and smoking

Table 12 *XRCC* genotypes smoking and risk for SCCA

Genotype	Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
	Cases	Controls		
Non-smokers				
XRCC1 codon 399				
Arg/Arg	12	27	1	1
Arg/Gln	8	14	1.28 [0.43- 3.88], 0.65	4.61 [0.47-45.04], 0.19
Gln/Gln	2	3	1.50 [0.22-10.17], 0.68	14.87 [0.69-317.9], 0.08
Arg/Gln+Gln/Gln	10	17	5.78 [0.64-52.32], 0.12	1.32 [0.47-3.72], 0.59
XRCC1 codon 194				
Arg/Arg	13	21	1	1
Arg/Trp	5	23	0.35 [0.11-1.15], 0.08	0.12 [0.01- 1.0], 0.06
Arg/Trp+Trp/Trp	9	23	0.33 [6.06-1.94], 0.22	0.63 [0.22- 1.78], 0.63
XRCC3 codon 241				
Thr/Thr	20	39	1	1
Thr/Met	2	5	0.7 8[0.14- 4.38], 0.78	3.84 [0.33-44.41], 0.28
Smokers				
XRCC1 codon 399				
Arg/Arg	54	42	1	1
Arg/Gln	33	30	0.85 [0.45- 1.61], 0.63	1.06 [0.46- 2.41], 0.89
Gln/Gln	2	2	0.77 [0.11-575], 0.81	1.07 [0.08-14.29], 0.96
Arg/Gln+Gln/Gln	35	32	1.06 [0.47- 2.38], 0.88	0.85 [0.45- 1.59], 0.61
XRCC1 codon 194				
Arg/Arg	40	44	1	1
Arg/Trp	44	28	1.73 [0.91- 3.27], 0.09	1.68 [0.40- 3.83], 0.21
Trp/Trp	5	2	2.75 [0.50-14.97], 0.24	2.39 [0.28-20.08], 0.42
Arg/Trp+Trp/Trp	49	30	1.74 [0.78- 3.87], 0.17	1.79 [0.96- 3.35], 0.06
XRCC3 codon 241				
Thr/Thr	81	67	1	1
Thr/Met	8	7	0.9 [0.3- 2.7], 0.92	1.5 [0.4- 6.3], 0.52

OR [95% CI] = Odds ratios [95%confidence interval], * adjusted with multiple logistic regression for age and HPV status

สรุปและวิจารณ์ผลการทดลอง

HPV infection is identified as a critical risk factor for the cervical cancer development in Northeast Thailand. Among high-risk HPV types, HPV-16 as well as other malignant types have more or less equal potential for the development of SCCA. The polymorphism of the *p53* itself as well as in combination with HPV infection may not be a genetic risk for cervical cancer. To other risk factors such as sexual behaviors and smoking, much attention should be paid. Since sexual behaviors, sexually transmitted diseases, the use of contraceptive and smoking may serve as cofactors to increase risk for cervical carcinoma in the presence of HPV. Eradication of HPVs by means of vaccination and/or lowering of HPV prevalence by education would be of great importance in this region.

Our research showed that passive tobacco smoking contributes to an increased risk of SCCA development among Northeast Thai women. Tobacco-related carcinogens in a smoking sex partner's seminal fluid were also applied directly to the cervix mucus membrane during sexual intercourse as they may play some role in the pathogenesis of cervical cancer.

The tobacco smoke constituents are modified by metabolizing enzymes and may promote malignant cellular growth. The mode of action is through the activation and detoxification of tobacco carcinogens; thus, one might expect the polymorphism of GSTs may alter the risk of cancer among smokers. The lack of GST activities caused by an inherited deletion of the *GST* have been reported to increase the risk of several tobacco-related cancers. (Kietthubthew et al., 2001;van der Hel et al., 2003;Sweeney et al., 2003; Lee et al., 2002). It was therefore hypothesized that smoking status and the *GST* genotype may synergistically influence cancer development. The effect of the *GST* null-genotype on the increased risk for cervical cancer among smokers was not observed in our study; even though the combination of the two GST null genotypes failed to increase the risk among subjects with exposure to tobacco smoke. Strong contributions of phase I detoxifying enzymes may mask the effects of GST null genotypes.

If the enzyme activity protects cancer development, the null alleles are deleterious and should be eliminated from the population by negative selective pressures. The high frequencies suggest that the lack of GST activity has unknown advantage(s) and maintains the persistence of these alleles in the population. Since (i) the substrate specificity of GSTs is relatively low, (ii) compensation of enzyme activity between GSTs may exist, and (iii) little exposure to tobacco smoke is expected in cervical cancer. These conditions may conceal the true influence of the null allele: their low specificity and bifunctional property also limits consistent interpretations throughout related cervical cancer studies, because the environmental conditions of each subjected population is different and the effects of/from this difference cannot be ruled out.

The relationships between the null-genotype for *GSTM1* and *GSTT1* and cancer susceptibility suggests a large-scale study with simultaneous analyses of phase I detoxifying enzyme genes. Currently, we just test for genotype or null-genotype presence; exact genotyping of wild-homozygous, heterozygous or null-homozygous, should be done in order to identify the cryptic effects of GST genotypes on the development of cervical cancer, with special reference to smoking status

The null genotype of phase I detoxification enzymes, GSTM1 and GSTT1, did not increase the risk for SCCA in smokers, moreover, the variant allele for DNA repair proteins, XRCC1 and XRCC3, do not increase the risk. It is implicated that modification or activation of pro-carcinogens/carcinogens by metabolizing enzymes may play critical roles in the development of cervical cancer. To reveal the role of phase II enzymes, such as CYP1 and CYP2 families, in the development of cervical cancer is strongly recommended.

Totally, this study indicates that variant types of DNA repair genes and phase I detoxification enzymes play partial roles in modifying individual susceptibility to cervical cancer. Since cervical cancer is a multi-factorial disease, the contribution of repair enzymes and detoxification enzymes if it ever exists to the development of the cervical cancer is concealed by the major risk factor, HPV infection, otherwise the increased risk should be found not only among HPV negative individuals but also HPV positive individuals.

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Output จากโครงการ

1. ผลงานการวิจัยที่ตีพิมพ์เผยแพร่ในวารสารระดับนานาชาติ

1.1 ได้รับการตีพิมพ์เผยแพร่แล้ว

-Settheetham-Ishida W, Yuenyao P, Kularbkaew C, Settheetham D, Ishida T. Glutathione S-transferase (GSTM1 and GSTT1) polymorphisms in cervical cancer in Northeastern Thailand. Asian Pac J Cancer Prev. 2009 10:365-8.

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1.2 กำลังรอการตีพิมพ์เผยแพร่ (ส่งไปเพื่อตีพิมพ์แล้ว)

-Settheetham-Ishida W, Yuenyao P, Settheetham D, Ishida T. Genetic risk of *XRCC1* and *XRCC3* polymorphism for cervical cancer in Northeast Thai. Gynecologic Oncology submission, manuscript number: GYN-09-1164

1.3 หนังสือ

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2. กิจกรรมอื่น ๆที่เกี่ยวข้อง ได้แก่ บทความ วิทยากร เสนอผลงาน

2.1 บทความ

-วรรณภา อิชิดะ. ภัยใกล้ตัวจากมะเร็งปากมดลูก. วารสารสำนักบริหารการวิจัย ปีที่ 2 ฉบับที่ 3 2550 หน้า 16-18.

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2.2 วิทยากร

-การสัมมนาวิชาการ JSPS-NRCT Joint Seminar 2007 ในหัวข้อเรื่อง Cervical Cancer in Northeastern Thailand วันที่ 30 พฤศจิกายน 2550 ณ. วิทยาลัยนานาชาติ มหาวิทยาลัยมหิดล ศาลายา

2.3 เสนอผลงาน

-เสนอผลงานวิจัยแบบโปสเตอร์ ประชุมนานาชาติ Infection-Immunity and Cancer ระหว่าง 19-21 กุมภาพันธ์ 2551ที่ โรงแรมเจริญธานี ขอนแก่น เรื่อง Glutathione S-transferases (*GSTM1* and *GSTT1)* and smoking habit in cervical cancer in Northeast Thailand

-เสนอผลงานวิจัยแบบปากเปล่าประชุมนานาชาติ HPV in Human Pathology ระหว่าง1-3พฤษภาคม 2551 ที่ เมือง ปราก ประเทศเช็ค เรื่อง Human Papillomavirus Infection in Cervical Abnormality in Northeast Thailand

-เสนอผลงานวิจัยแบบโปสเตอร์ ประชุมประชุมวิชาการสรีรวิทยาสมาคมแห่งประเทศไทยครั้งที่ 38 ประจำปี 2552 "Active Good Health: Physiology and Alterative Medicine" ระหว่าง 1-3 เมษายน 2552 ที่ โรงแรมอิพีเรียล ภูแก้วฮิลล์ จ.เพชรบูรณ์ เรื่อง Molecular epidemiology of DNA repair gene and cervical cancer susceptibility

ภาคผนวก

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Glutathione S-transferase (GSTM1 and GSTT1) polymorphisms in cervical cancer in Northeastern Thailand.

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To evaluate the relationships between genetic polymorphisms of the GSTs (GSTM1 and GSTT1) and cervical cancer, the null genotype of each gene was studied in squamous cell cervical cancer (SCCA) patients (n=90) and controls (n=94) in Northeast Thailand. The prevalence of the GSTM1-null genotype in the controls and SCCA patients was 59.6% and 60.0%, respectively, whereas those of the GSTT1-null genotype in the control and SCCA patients was 40.4% and 46.7%, respectively. Neither of the GST-null genotypes increased the risk for SCCA ($p>0.05$); however, the combination of the GSTM-1 and GSTT1-null genotypes showed a non-significant trend for an increased risk for developing cervical cancer with an adjusted OR of 2.7 (95%CI=0.8-9.0, $p=0.10$). Genetic polymorphisms of GSTM1 and GSTT1 were not significant risk factors for cervical cancer in either tobacco-smokers or non-smokers. A different contribution of the GST genotype to cancer risk may be attributed to a different, as yet undefined, property of the enzymes.

Glutathione S-transferases (*GSTM1* and *GSTT1*) polymorphism in cervical cancer of Northeastern Thailand

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Keywords: Cervical cancer, *GSTM1*, *GSTT1*, Northeastern Thailand, Genetic susceptibility

Abstract

To evaluate the relationships between genetic polymorphisms of the *GSTs* (*GSTM1* and *GSTT1*) and cervical cancer, the null genotype of each gene was studied in squamous cell cervical cancer (SCCA) patients (n=90) and controls (n=94) in Northeast Thailand. The prevalence of the *GSTM1*-null genotype in the controls and SCCA patients was 59.6% and 60.0%, respectively, whereas those of the *GSTT1*-null genotype in the control and SCCA patients was 40.4% and 46.7%, respectively. Neither of the *GST*-null genotypes increased the risk for SCCA ($p>0.05$); however, the combination of the *GSTM-1* and *GSTT1*-null genotypes showed a trend to an increased risk for developing cervical cancer with adjusted OR=2.7 (95%CI=0.8-9.0, $p=0.10$). Genetic polymorphism of GSTM1 and GSTT1 was not a significant risk for cervical cancer in either tobacco-smokers or non-smokers. A different contribution of the *GST* genotype to cancer risk may be attributed to a different, as yet undefined, property of the enzymes.

Introduction

Cervical cancer remains a national health problem in Thailand. The principal causative factor for the development of cervical cancer is human papillomavirus (HPV) infection and the prevalence of HPV infection in Northeast Thai women is high (Vatanasapt et al., 1995; Settheetham-Ishida et al., 2005). Notwithstanding, only a small proportion of HPV carriers develop cervical cancer, indicating some other factor(s) responsible for the development of cervical cancer. It is widely reported that tobacco smoking increases the risk for many types of cancer including cervical cancer (Prokopczyk et al., 1997; Parkin et al., 1994; Simons et al., 1993). Carcinogens, such as nicotine, cotinine and tobacco-specific nitrosamines, have been detected in the cervical mucus of smokers (McCann et al., 1992). And yet, even though inhaled tobacco-derived components may damage smokers' cervical cellular DNA, not all smokers develop cervical cancer (Prokopczyk et al., 1997; Ballinger et al., 1996). The difference, therefore, in the metabolic efficiency of tobacco smoke pro-carcinogens is thought to be the individual's susceptibility to cervical cancer.

Glutathione S-transferase (GST) is related to human phase II detoxification enzymes. Cytosolic GSTs (GSTM, GSTP and GSTT) play key roles in the detoxification of the carcinogenic electrophiles of aflatoxin and polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke (benzo[*a*]pyrene and other PAH procarcinogens). The mode of action of GSTs is through the activation and detoxification of tobacco carcinogens; therefore, one might expect to find a relationship between the genetic polymorphisms of GSTs and the risk of developing cancer (Heagerty et al., 1994; Nazar-Stewart et al., 1999; Setiawan et al., 2000; Sweeney et al., 2003; Tiwawech et al., 2005).

GSTM1 facilitates the excretion of a wide range of carcinogens, reactive oxygen species and chemotherapeutic agents with a variety of substrate specificities (Rebbeck, 1997). GSTT1 is also involved in the detoxification of environmental carcinogens such as 1,3 butadiene and ethylene oxide in tobacco smoke and ambient air (Landi, 2000). The absence of a homozygous allele in the *GST* (*GST*-null genotype) results in a complete loss of enzyme activity to bind with genotoxic substrates, including epoxides derived from aflatoxin and PAHs (Hayes and Pulford, 1995). Individuals with *GSTM1*-null or *GSTT1*-null genotype have been investigated as to whether they were susceptible to various cancers including lung, bladder, skin, oral, liver, gastric, colorectal, prostrate, breast, ovary, cervix and nasopharynx (Nazar-Stewart et al., 1999; Sweeney et al., 2003; Lee et al., 2002; Heagerty et al., 1994; Kietthubthew et al., 2001; Deng et al., 2001; Setiawan et al., 2000; Gawronska-Szklarz et al., 1999; Autrup et al., 1999; van der Hel et al., 2003; Spurdle et al., 2001; and Sierra-Torres et al., 2003), but the interpretation of the results was not consistent.

In a previous study, we found that smoking was a critical risk factor for the development of cervical cancer (Setteheetham-Ishida et al., 2004) and it is possible that phase II detoxification enzymes play roles in this development. The frequency of the *GST*-null genotype differs by population (Kietthubthew et al., 2001;Tiwawech et al., 2005) and the data on GST polymorphism in Thai cervical cancer are missing. We thus designed our study to

investigate the *GSTM1*- and *GSTT1*-null genotypes and their susceptibility to cervical cancer among women in Northeast Thailand.

Materials and Methods

Subjects

Women between 27 and 74 years of age attending Srinagarind Hospital, Khon Kaen University, Thailand, were recruited. Cases (n=90) were defined as squamous cell cervical cancer (SCCA) with cytological, colposcopical and histological diagnosis. Controls (n=94) were recruited from healthy women without cervical cancer, history of conization, hysteretomy or diseases associated with known risk factors for cervical cancer. The subjects were pooled; cases and controls were matched by 5-year age classes then divided into groups according to their smoking status. Prior to this study, the subjects were examined for p53 codon 72 polymorphism (Settheetham-Ishida et al., 2004) and HPV infection (Settheetham-Ishida et al., 2005). The patients were informed of the purpose and experimental procedures of the study and written informed consent obtained. This study was approved by the Ethics Committee of Khon Kaen University.

GSTM1 and *GSTT1* genotyping

DNA was extracted from peripheral blood cells. The *GSTM1* and *GSTT1* genotypes were determined using PCR methods. The primers for the *GSTM1* genotype were 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and 5'-GTT GGG CTC AAA TAT ACG GTG G-3', and for the *GSTT1* genotype 5'-TTC CTT ACT GTC CTC ACA TCT C-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3'. Co-amplification of the *human β-globin* using primers 5'- AAC TTC ATC CAC GTT CAC C-3' and 5'-GAA GAG CCA AGG ACA GGT AC -3' was used to confirm the true *GSTM1*- and GSTT1-null genotype as opposed to a failure in the PCR assays. Only samples that gave a *β-globin* PCR positive result were recruited. The PCR products were electrophoresed on 2.5% agarose gel and visualized with ethidium bromide staining. The PCR product of *GSTM1, GSTT1* and *β -globin* was 215, 408 and 268 base pairs in length, respectively.

Statistical analyses

The χ^2-test was used to compare the genotype frequency of *GSTM1* or *GSTT1* polymorphism between the cervical carcinoma patients and the controls. Associations between the *GST* genotypes and the risk of cervical cancer were tested using odds ratios and 95% confidence intervals (OR and 95% CI), calculated by multivariate logistic regression analysis with 800-STATA-PC. A *P*-value <0.05 was considered significantly different.

Results

Table 1 shows the genotype distribution of *GSTM1 and GSTT1* for the cases and controls. The prevalence of the *GSTM1*-null genotype in the control and SCCA patients was 59.6% and 60.0%, respectively, whereas that of the *GSTT1*-null genotype in the control and SCCA patients was 40.4% and 46.7%, respectively. Neither the *GSTM1*- nor *GSTT1*-null genotypes increased the risk for SCCA (p>0.05). The combination of the *GSTM1*- and *GSTT1*-null genotypes showed a trend to increasing the risk of developing cervical cancer with an adjusted OR of 2.72 (95%CI=0.82-9.03, p= 0.10). This trend was also observed when we tested smokers (Table 2); namely, the *GSTM1*- or *GSTT1*-null genotype did not increase the risk for SCCA in smokers (p>0.05), whereas an increased risk for SCCA with an adjusted OR=1.82 (95%CI=43-7.67, p=0.41) was observed in the *GST* double negative carriers.

Table 1 Genetic polymorphism of *GSTM1* and *GSTT1* in the cervical cancer

Genotypes	**Subjects**, n(%)		OR	Adjusted OR*
	Cases	Controls	[95%CI,p-value]	[95%CI,p-value]
GSTM1				
+	36(40.0)	38(40.4)	1	
-	54(60.0)	56(59.6)	1.02 [0.54-1.91,0.95]	0.66 [0.30-1.48, 0.32]
GSTT1				
+	48(53.3)	56(59.6)	1	
-	42(46.7)	38(40.4)	1.29 [0.69-2.41,0.39]	0.72 [0.29-1.80, 0.48]
GSTM1/T1				
+/+	20(22.2)	18(19.1)	1	
+/-	28(31.1)	38(40.4)	0.67 [0.35-1.78, 0.19]	-**
-/+	16(17.8)	20(21.3)	0.80 [0.38-1.77, 0.55]	-**
-/-	26(28.9)	18(19.1)	1.72 [0.82-3.64, 0.12]	2.72 [0.82-9.03, 0.10]

OR [95% CI] = odds ratios [95% confidence interval]

* Adjusted for age, *p53* genotypes, smoking and HPV status

**drop because of co-linearity

Table 2 GST genotypes and risk for SCCA among smokers

Genotypes	**Subjects**, n(%)		OR	Adjusted OR*
	Cases	Controls	[95%CI,p-value]	[95%CI,p-value]
GSTM1				
+	30(41.1)	24(42.1)	1	1
-	43(58.9)	33(57.9)	1.00	0.78
			[0.46-2.15, 1.00]	[0.30-2.03, 0.62]
GSTT1				
+	39(53.4)	34(59.6)	1	1
-	34(46.6)	23(40.4)	1.35	1.74
			[0.63-2.91, 0.43]	[0.68-4.45, 0.25]
GSTM1/T1				
+/+	16(21.9)	12(21.4)	1	1
+/-	23(31.5)	22(39.3)	0.71	-**
			[0.32-1.57, 0.36]	
-/+	14(19.2)	11(19.6)	0.97	-**
			[0.37-2.61, 0.95]	
-/-	20(27.4)	11(19.6)	1.54	1.82
			[0.62-3.96, 0.31]	[0.43-7.67, 0.41]

OR [95% CI] = Odds ratios [95%confidence interval]

* Adjusted for age, *p53* genotypes and HPV status

**drop because of collinearity

Discussion

The underlying background for the present and related studies is that the enzyme activity of GSTs in detoxification protects from cancer development, which results in the higher cancer incidence with *GST*-null genotypes. However, not every *GSTM1*- or *GSTT1*-null genotype increased the risk for SCCA ($p>0.05$). These observations are in agreement with studies among Caucasians (Warwick et al., 1994b, Warwick et al., 1994a, Chen et al., 1999, Goodman et al.,2001), Indians (Sharma et al.,2004; Sobti et al, 2006), and Japanese (Niwa et al., 2005); which reported no difference in the frequency of the *GSTM1*- and *GSTT1*-null genotypes between the controls and cervical carcinoma cases.

There are controversial reports on women carrying both the *GSTT1* and *GSTM1* null genotypes having an increased risk for cervical carcinoma among Koreans (Kim et al., 2000) and a risk for high-grade cervical neoplasia and invasive cervical cancers among Caucasians (Sierra-Torres et al.,2003). These controversial results are frequently observed in studies on GST and cancer susceptibility. It is interesting that persons who carry both the *GSTM1* and *GSTT1* null genotype showed a trend for an increased risk of SCCA (Table 1 and 2). As phase II detoxifying enzymes, GSTM1 and GSTT1 have no stringent substrate spectra and different contributions of *GST* null-genotype to the risk of cancer; perhaps, then, it is attributable to the different properties between GSTM1 and GSTT1.

Our previous research showed that passive tobacco smoking contributes to an increased risk of SCCA development among Northeast Thai women (Ishida et al., 2004). Tobacco-related carcinogens in a smoking sex partner's seminal fluid were also applied directly to the cervix mucus membrane during sexual intercourse as they may play some role in the pathogenesis of cervical cancer (Kulikauskas et al., 1985; Lahdetie et al., 1986).

The tobacco smoke constituents are modified by metabolizing enzymes and may promote malignant cellular growth (Prokopczyk et al., 1997). The mode of action is through the activation and detoxification of tobacco carcinogens; thus, one might expect the polymorphism of GSTs may alter the risk of cancer among smokers. The lack of GST activities caused by an inherited deletion of the *GST* have been reported to increase the risk of several tobacco-related cancers (Heagerty et al., 1994; Gawronska-Szklarz et al., 1999; Autrup et al., 1999; Nazar-Stewart et al., 1999; Setiawan et al., 2000;Deng et al., 2001;Spurdle et al., 2001; Kietthubthew et al., 2001;van der Hel et al., 2003;Sweeney et al., 2003; Lee et al., 2002). It was therefore hypothesized that smoking status and the *GST* genotype may synergistically influence cancer development. In India, the absence of the *GSTM1 and GSTT1* gene increased the risk of cervical cancer among passive smokers 7.0- and 10.2-fold, respectively (Sobti et al., 2006). No significant interaction was found between tobacco smoking and the genetic background of GSTM1 on the risk of cervical squamous intraepithelial lesion in Hawaii (Goodman et al., 2001). The effect of the *GST* null-genotype on the increased risk for cervical cancer among smokers was not observed in our study; even though the combination of the two GST null genotypes failed to increase the risk among

subjects with exposure to tobacco smoke. Strong contributions of phase I detoxifying enzymes may mask the effects of GST null genotypes.

The prevalence of the null genotypes for the *GSTM1* (0.60) and *GSTT1* (0.40) among Northeast Thais was comparable to the results reported for other Thais; in the Central region (0.60 and 0.38) (Tiwawech et al., 2005; Pakakasama et al., 2005) and the South (0.66 and 0.36) (Kietthubthew et al., 2001) and other Asian populations. The high prevalence of the null genotypes may, therefore, give a clue to explaining the controversial results.

If the enzyme activity protects cancer development, the null alleles are deleterious and should be eliminated from the population by negative selective pressures. The high frequencies suggest that the lack of GST activity has unknown advantage(s) and maintains the persistence of these alleles in the population. Since (i) the substrate specificity of GSTs is relatively low, (ii) compensation of enzyme activity between GSTs may exist, and (iii) little exposure to tobacco smoke is expected in cervical cancer. These conditions may conceal the true influence of the null allele: their low specificity and bifunctional property also limits consistent interpretations throughout related cervical cancer studies, because the environmental conditions of each subjected population is different and the effects of/from this difference cannot be ruled out.

The relationships between the null-genotype for *GSTM1* and *GSTT1* and cancer susceptibility suggests a large-scale study with simultaneous analyses of phase I detoxifying enzyme genes. Currently, we just test for genotype or null-genotype presence; exact genotyping of wild-homozygous, heterozygous or null-homozygous, should be done in order to identify the cryptic effects of GST genotypes on the development of cervical cancer, with special reference to smoking status.

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TP53 codon 72 polymorphism and cervical cancer: a pooled analysis of individual data from 49 studies

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Summary

Background Cervical cancer is caused primarily by human papillomaviruses (HPV). The polymorphism rs1042522 at codon 72 of the *TP53* tumour-suppressor gene has been investigated as a genetic cofactor. More than 80 studies were done between 1998 and 2006, after it was initially reported that women who are homozygous for the arginine allele had a risk for cervical cancer seven times higher than women who were heterozygous for the allele. However, results have been inconsistent. Here we analyse pooled data from 49 studies to determine whether there is an association between *TP53* codon 72 polymorphism and cervical cancer.

Methods Individual data on 7946 cases and 7888 controls from 49 different studies worldwide were reanalysed. Odds ratios (OR) were estimated using logistic regression, stratifying by study and ethnic origin. Subgroup analyses were done for infection with HPV, ethnic origin, Hardy–Weinberg equilibrium, study quality, and the material used to determine *TP53* genotype.

Findings The pooled estimates (OR) for invasive cervical cancer were 1·22 (95% CI 1·08–1·39) for arginine homozygotes compared with heterozygotes, and 1·13 (0·94–1·35) for arginine homozygotes versus proline homozygotes. Subgroup analyses showed significant excess risks only in studies where controls were not in Hardy–Weinberg equilibrium (1·71 [1·21–2·42] for arginine homozygotes compared with heterozygotes), in non-epidemiological studies (1·35 [1·15–1·58] for arginine homozygotes compared with heterozygotes), and in studies where *TP53* genotype was determined from tumour tissue (1·39 [1·13–1·73] for arginine homozygotes compared with heterozygotes). Null results were noted in studies with sound epidemiological design and conduct (1·06 [0·87–1·29] for arginine homozygotes compared with heterozygotes), and studies in which *TP53* genotype was determined from white blood cells (1·06 [0·87–1·29] for arginine homozygotes compared with heterozygotes).

Interpretation Subgroup analyses indicated that excess risks were most likely not due to clinical or biological factors, but to errors in study methods. No association was found between cervical cancer and *TP53* codon 72 polymorphism when the analysis was restricted to methodologically sound studies.

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Introduction

Cervical cancer is the third most frequent cause of death from cancer among women worldwide.[1] Human papillomaviruses (HPV) are a necessary but insufficient cause of cervical cancer,[2,3] and infection with HPV is very common among young sexually active women.[4] Although the immune systems of healthy women clear the infection within 1–2 years, persistent infection with oncogenic HPV types can lead to high-grade cervical intraepithelial lesions and, if untreated, to cervical cancer.[4]

In 2006, the first vaccine against HPV was approved by the US Food and Drug Administration and the European Medicines Agency. Cervical cancer develops over many years,[4] during which time precancerous lesions can be detected by cytological screening with the Papanicolaou smear and removed surgically, if indicated. However, many women around the world do not have access to HPV vaccination or cytological screening.

Most HPV-infected women do not develop cervical cancer,[4] so the identification of cofactors would make it possible to adapt prevention strategies to particular risk groups. The cofactors so far confirmed in large meta-analyses are smoking, long-term use of oral contraceptives, and increased parity.[5–7] No evidence of an association with genetic cofactors has yet been found in large analyses, but certain human leucocyte antigen (HLA) genotypes might increase the risk of cervical cancer.[8] Over the past decade, a polymorphism at codon 72 of the *TP53* gene (Pro72Arg; rs1042522) has been investigated. Three genotypes occur: arginine homozygotes, proline

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homozygotes, and heterozygotes.[9] A guanine/cytosine variant at the second position of codon 72 on exon 4 leads to Arg72 or Pro72 protein variants with markedly altered primary structures and different biochemical functions.[9–11] The current view is that the P53–Arg72 protein is more effective at inducing apoptosis and protecting cells from tumour development than the P53–Pro72 protein.[9,12] Various cell-culture studies have reported differences in mitochondrial localisation, cell-cycle progression, DNA repair, growth arrest, and transcriptional activation.[12] However, there are insufficient experimental data to establish whether there are consistent differences in biological activity between the two protein variants.[12]

The P53 tumour-suppressor protein has been called the guardian of human cells against cancer.[13,14] The E6 oncogene of HPV has been shown to bind to cellular P53 and promote its degradation,[15,16] and it is thought that the Arg72 variant might be more susceptible to this degradation than the Pro72 variant.[10]

A study published in 1998 showed that women homozygous for arginine at codon 72 of the *TP53* tumour-suppressor gene had a risk for cervical cancer seven times that of heterozygous women.[17] Subsequently, more than 80 studies have been published on this issue, with widely inconsistent results. Only one study quantitatively supported the initial findings of Storey and colleagues,[18] other studies also found an increased risk,[19–21] while many did not.[22–26] Two meta-analyses based on early studies and published data only found significant associations with squamous-cell carcinoma[27] or adenocarcinoma.[28] Concern was expressed about methodological issues, such as departure from Hardy–Weinberg equilibrium.[22,27,29] It is therefore clear that the findings so far are inconsistent, there is a paucity of large studies with appropriate statistical power, and it remains unclear whether there is an association between *TP53* codon 72 polymorphism and cervical cancer. To address these issues, we did a pooled analysis of original individual data from 49 studies, with the aim of resolving conclusively the question of whether there is an association between *TP53* codon 72 polymorphism and cervical cancer.

Methods

Literature search

We searched various databases including PubMed, Embase, and Current Contents to identify studies on *TP53* polymorphism and cervical cancer published before 2007. Additionally, we searched the internet for unpublished data. All studies on *TP53* polymorphism and cervical cancer published before 2007 were included. No language restrictions were applied; all non-English articles were translated if necessary. Interim analyses, overlapping study populations, and comparisons of laboratory methods were excluded. For unpublished studies, data collection had to have ended in 2006.

Procedures

The corresponding and first authors of the published studies and the principal investigators of unpublished research were contacted and asked to provide their original data. Details of study conduct and data coding were elicited by a short questionnaire. The data transmitted included *TP53* genotype, cervical diagnosis, age, sex, ethnic group, HPV status, and the methods and materials used for *TP53* genotyping and HPV detection. All data were recoded according to an a-priori protocol and combined into a common database. Men (n=11), girls under 15 (n=59), and participants with unknown *TP53* status or unclear case or control status were excluded.

Potential publication bias was evaluated using the Egger's test,[30] with p values below 0·05 considered statistically significant. Heterogeneity of genotype effects was tested using the score test for interaction between study and genotype within a logistic model,[31] with p values below 0·05 considered statistically significant. All p values were two-sided.

Cases of invasive cervical cancer were confirmed histologically, while high-grade and low-grade lesions were confirmed by either histology or cytology. The group of all invasive cervical cancers consisted of squamous-cell carcinoma, adenocarcinoma, adenosquamous carcinoma, and unknown types. The group of high-grade lesions consisted of high-grade squamous intraepithelial lesions and cervical intraepithelial lesions grades 2 and 3. Low-grade lesions consisted of low-grade squamous intraepithelial lesions and cervical intraepithelial lesions grade 1. Only controls confirmed by negative cytology were used in analyses.

Four criteria were used to evaluate study quality: Hardy–Weinberg equilibrium among controls, study type, study size, and source of material used to determine *TP53* genotype. Deviations from Hardy–Weinberg equilibrium for controls were calculated with the permutation version of the exact test (SAS version 9.1), and p values below 0·05 were considered to represent significant violation of Hardy–Weinberg equilibrium.[32] Study type was defined as either epidemiological or non-epidemiological. For a study to be considered epidemiological, four criteria had to be met for the entire study population: case and control status confirmed by histology or cytology; ages of cases and controls known; cases and controls recruited from the same base population; and no case series. Study size was divided into those with at least 200 participants and those with fewer than 200 participants. Studies using white blood cells, exfoliated cells, or tumour tissue to extract DNA to determine *TP53* genotype were analysed separately.

Statistical analysis

Pooled estimates and 95% CI were calculated by logistic regression with SAS version 9.1. The *TP53* codon 72 genotype was analysed as one variable with three categories: arginine homozygotes (Arg/Arg) or heterozygotes (Arg/Pro) versus proline homozygotes

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(Pro/Pro, reference) and Arg/Arg versus Arg/Pro (reference). Additionally, the *TP53* genotype was dichotomised for Arg/Arg versus Arg/Pro and Pro/Pro (reference). Odds ratios (OR) were also calculated for all arginine alleles versus all proline alleles (reference), with each individual considered twice. In all logistic regression models, only studies that contributed at least five cases and five controls were included. Analyses were stratified by study to ensure that comparisons were only made between cases and controls of the same study. It is known that the *TP53* polymorphism varies between ethnic groups.[33] In the individual studies included in this pooled analysis only one ethnic group per study was found in all but two studies,[22,34] in which case we additionally classed the different ethnic groups as separate studies to ensure that comparisons were only made between cases and controls of the same ethnic group and the same study.

We did not adjust for potential risk factors such as age and HPV status. *TP53* genotype status in cases is independent of age, and there were too many missing values (35%). Adjusting by study and age would have led to an uncontrolled selection of studies contributing sufficient cases and controls, which would have biased the results of our analysis. HPV status was accounted for by subgroup analyses, since several studies failed to contribute sufficient HPV-negative cases or HPV-positive controls. This imbalance rendered HPV-adjusted models impossible.

Studies or individual women that were missing information on any variable considered in any analyses were included by generating a "missing" category for the variable. To investigate the significance of effect between genotypes, a Wald χ^2 test with two degrees of freedom was done.[31] p values were two-sided and considered statistically significant when below 0·05. In subgroup analyses p values were Bonferroni adjusted to account for multiple testing, and were considered statistically significant below 0·025.

The study population was stratified by tumour and cervical histology, HPV status, ethnic group, Hardy–Weinberg equilibrium in controls, study type, study size, and the material used to determine the *TP53* genotype. All subgroup analyses with the exception of the subgroup of cervical histology included only invasive cancer cases. Effect modification by subgroup was assessed by testing the interaction between genotype and stratification variable within the logistic model.[31] Subgroup analyses were determined a priori in the statistical analysis plan. However, subgroup analyses were considered to be of exploratory nature.[35]

For sensitivity analyses, a complete case analysis was done on cases and controls for which there were no missing values for age, HPV, or ethnic group. A dataset was then used that comprised only epidemiological studies with controls in Hardy–Weinberg equilibrium

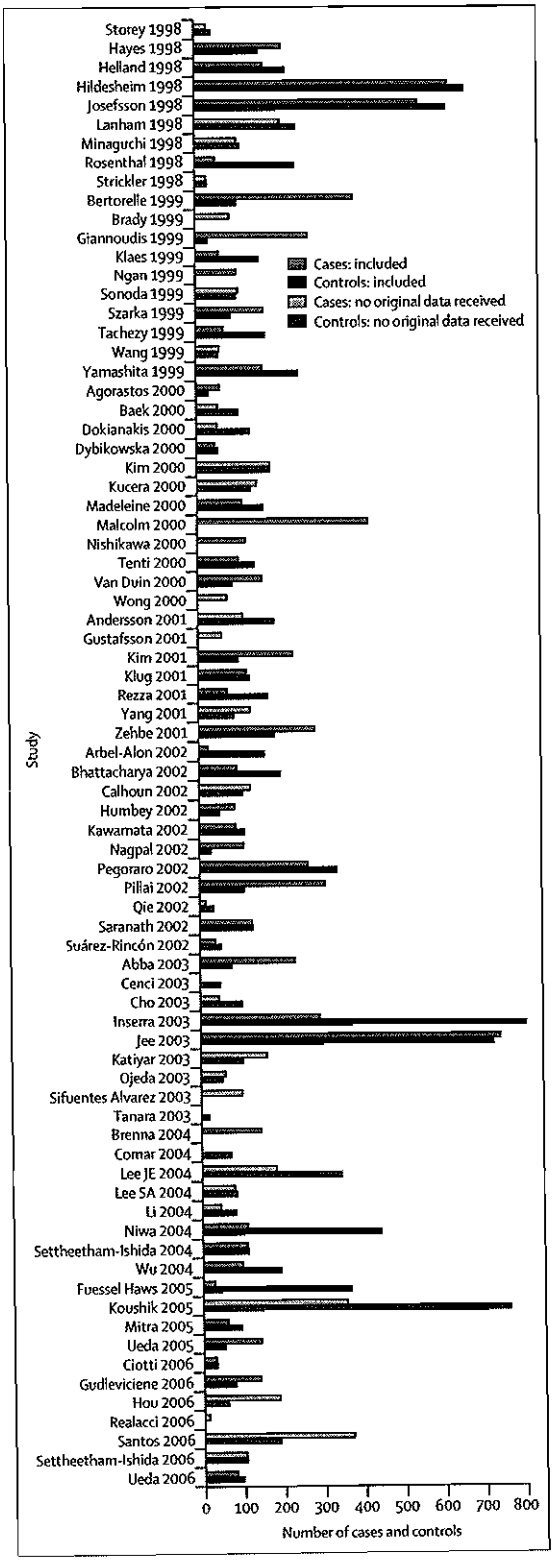

Figure 1: **Cases and controls of 48 published studies included in the pooled reanalysis with individual data**
Cases (green) and controls (red) used in the analysis and cases (light blue) and controls (dark blue) in studies for which data were not available.

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and *TP53* genotype detection from white blood cells. An analysis was then done with a dataset made up of studies that used white blood cells to determine *TP53* genotype.

Role of the funding source

The sponsor of this study had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report. SJK, MR, JK, and MB had full access to all of the raw data that were made available for this pooled analysis. The corresponding author had full access to all of the data that were made available for this pooled analysis and had the final responsibility for the decision to submit the manuscript for publication.

Results

We identified 87 articles published between 1998 and 2006, and five unpublished studies. Of the published articles, ten did not fit the inclusion criteria. The authors of the remaining 77 articles were contacted, and 45 investigators (58%) provided original individual data on participants in 48 studies (figure 1). Additionally, one group provided unpublished data (Schmitt VM, unpublished). Therefore, 49 studies from 26 countries were included in the pooled analysis (table 1). Original data collection within individual studies took place between 1969 and 2005. Egger's test was done to investigate publication bias; p values for comparison of Arg/Arg versus Arg/Pro, Arg/Arg versus Pro/Pro, and Arg/Pro versus Pro/Pro were 0·008, 0·36, and 0·98, respectively.

Most of the studies had been done in Europe (19) and Asia (17); 12 had been done in the Americas and one study in Africa. Two cohort studies, four cross-sectional studies, 40 case–control studies, and three case series were included. The material used for *TP53* genotyping, from both cases and controls, was white blood cells or exfoliated cervical cells in 26 (53%) of the studies: the remaining 23 (47%) of studies used tumour tissue from cases (table 2). The controls of 27 studies (55%) were in Hardy–Weinberg equilibrium. Half the studies were large, with 200 or more participants (51%), but only 16 studies (33%) fulfilled the criteria of sound epidemiological design and conduct. The study-specific genotype effects were analysed for each study, and no statistically significant heterogeneity was noted between studies.

TP53 codon 72 genotype was available for 15 834 women (7946 cases and 7888 controls), for whom case or control status was confirmed by histology or cytology (table 3). Most cases and controls were white women. HPV infection was present in 5095 (64%) of cases and 1353 (17%) of controls; however, HPV infection status was not

	Case/control	Study type	Year of data collection	Country	Ethnic group	Material used for assessment of *TP53* genotype	Controls in Hardy-Weinberg equilibrium
1998							
Hayes and colleagues[29]	215/158*	Non-epidemiological study	1995–96	Netherlands	White	White blood cells	Yes
Helland and colleagues[36]	169/225	Epidemiological study (case–control)	1991–92 (CIN, controls), 1989–91 (cervical cancer)	Norway	White	White blood cells	Yes
Hildesheim and colleagues (Costa Rica)[24]	147/205	Epidemiological study (cohort)	1993–94	Costa Rica	Hispanic	White blood cells	Yes
Hildesheim and colleagues (Portland, USA)[24]	208/217	Epidemiological study (cohort)	1987–89	USA	85% white; 2% black; 13% unknown	Exfoliated cervical cells	Yes
Hildesheim and colleagues (east USA)[24]	270/245*	Non-epidemiological study	1992–96 (cases), 1994–96 (controls)	USA	84% white; 2% asian; 9% black; 4% hispanic; 1% unknown	Exfoliated cervical cells	Yes
Josefsson and colleagues[25]	551/621	Epidemiological study (case–control)	1969–99	Sweden	White	Exfoliated cervical cells	No
Rosenthal and colleagues[37]	50/246*	Non-epidemiological study	1998	Great Britain	White	Tumour tissue (cases)	Yes
1999							
Bertorelle and colleagues[38]	390/102	Non-epidemiological study	1998	Italy	White	Exfoliated cervical cells	Yes
Giannoudis and colleagues[39]	279/30	Non-epidemiological study	1996–98 (cases), 1998 (controls)	Great Britain	White	Tumour tissue (cases)	Yes
Klaes and colleagues[40]	58/156	Non-epidemiological study	NK	Germany	White	Mixed (tissue or exfoliated cervical cells)	Yes
Ngan and colleagues[41]	102/0†	Non-epidemiological study	1990–94 (cases, controls NK)	China	Asian	Tumour tissue (cases)	NA
Szarka and colleagues[42]	168/87*	Non-epidemiological study	NK	Hungary	White	Tumour tissue (cases), white blood cells (controls)	Yes
Tachezy and colleagues[23]	68/171	Epidemiological study (case–control)	1993–97 (cases), 1993–98 (controls)	Czech Republic	White	White blood cells	Yes
Yamashita and colleagues[43]	164/252	Non-epidemiological study	1990–97	Japan	Asian	Tumour tissue (cases), exfoliated cervical cells (controls)	Yes

(Continues on next page)

	Case/control	Study type	Year of data collection	Country	Ethnic group	Material used for assessment of *TP53* genotype	Controls in Hardy-Weinberg equilibrium
(Continued from previous page)							
2000							
Agorastos and colleagues[18]	58/30	Non-epidemiological study	1996–97 (cases), 1999 (controls)	Greece	White	Tumour tissue (cases), exfoliated cervical cells (controls)	Yes
Dybikowska and colleagues[44]	44/52*	Non-epidemiological study	1995–99	Poland	White	Exfoliated cervical cells	Yes
Madeleine and colleagues[26]	111/164*	Non-epidemiological study	1986–97	USA	White	White blood cells	Yes
Malcolm and colleagues[45]	423/0	Non-epidemiological study	1996–99	USA	Unknown	Tumour tissue (cases)	NA
Nishikawa and colleagues[46]	119/0	Non-epidemiological study	1984–94	Japan	Asian	Tumour tissue (cases)	NA
Tenti and colleagues[47]	101/140	Epidemiological study (case-control)	1990–98 (cases), 1995–96 (controls)	Italy	White	Tumour tissue (cases), exfoliated cervical cells (controls)	Yes
Van Duin and colleagues[48]	160/86	Non-epidemiological study	NK	Netherlands	White	Mixed: tumour tissue (some cases), exfoliated cervical cells (controls and some cases)	Yes
2001							
Kim and colleagues[49]	234/100	Epidemiological study (case-control)	1997–98	Korea	Asian	Mixed: white blood cells, tissue (some cases)	Yes
Klug and colleagues[21]	119/127	Epidemiological study (case-control)	1996–97	Peru	89% hispanic, 7% white, 3% black, 1% asian	White blood cells	Yes
Rezza and colleagues[50]	71/172	Epidemiological study (cross-sectional)	1995–96	Italy	White	Exfoliated cervical cells	Yes
Zehbe and colleagues (Italy)[19]	111/0†	Non-epidemiological study	1996–98	Italy	White	Tumour tissue (cases)	NA
Zehbe and colleagues (Sweden)[19]	177/188	Non-epidemiological study	1990–99	Sweden	White	Exfoliated cervical cells (controls), tumour tissue (cases)	Yes
2002							
Arbel-Alon and colleagues[51]	23/162*	Non-epidemiological study	1995–96	Israel	White	White blood cells	No
Bhattacharya and colleagues[52]	93/201	Epidemiological study (case-control)	1997 (cases), 1997–99 (controls)	India	Asian	Tumour tissue (cases), exfoliated cervical cells (controls)	Yes
Humbey and colleagues[53]	88/50	Epidemiological study (cross-sectional)	1997–2001	France	White	Exfoliated cervical cells	No
Kawamata and colleagues[54]	90/112	Non-epidemiological study	NK	Japan	Asian	Tumour tissue (cases), exfoliated cervical cells (controls)	Yes
Nagpal and colleagues[55]	110/29	Non-epidemiological study	NK	India	Asian	Tumour tissue (cases)	Yes
Pegoraro and colleagues[23]	269/340*	Non-epidemiological study	1998–2000	South Africa	Black	Mixed: white blood cells, tumour tissue (some cases)	Yes
Pillai and colleagues[56]	311/110	Non-epidemiological study	2000–02	India	Asian	Mixed: white blood cells, tissue	Yes
Saranath and colleagues[57]	130/131	Non-epidemiological study	1999–2000	India	Asian	Mixed: white blood cells, tissue	No
Suárez-Rincón and colleagues[58]	38/52	Non-epidemiological study	1999–2000	Mexico	Hispanic	Tumour tissue (cases)	No
2003							
Abba and colleagues[59]	235/79	Non-epidemiological study	1990–2002 (cases) 1998–2002 (controls)	Argentina	Hispanic	Tumour tissue (cases)	Yes
Inserra and colleagues[24]	295/2903	Epidemiological study (cross-sectional)	1992–99	USA and Mexico	61% hispanic; 35% white; 2% black; 1% asian; 1% unknown	Exfoliated cervical cells	Yes
Jee and colleagues[60]	741/724	Non-epidemiological study	2000–01	Korea	Asian	White blood cells	Yes
2004							
Brenna and colleagues[61]	148/0	Non-epidemiological study	1992–2002	Brazil	Mixed	Tumour tissue (cases)	NA
Niwa and colleagues[62]	112/442*	Non-epidemiological study	2001–03 (cases), 1999–2000 (controls)	Japan	Asian	White blood cells	Yes
Settheetham-Ishida and colleagues[63]	111/114	Epidemiological study (case-control)	2002–03	Thailand	Asian	White blood cells	Yes
Wu and colleagues[64]	99/193	Epidemiological study (case-control)	1999–2000	Taiwan	Asian	White blood cells	Yes

(Continues on next page)

	Case/control	Study type	Year of data collection	Country	Ethnic group	Material used for assessment of *TP53* genotype	Controls in Hardy-Weinberg equilibrium
(Continued from previous page)							
2005							
Haws and colleagues[65]	29/367	Epidemiological study (cross-sectional)	2002–04	USA, Mexico	Hispanic	Exfoliated cervical cells	No
Mitra and colleagues[66]	61/94*	Non-epidemiological study	1997–2003	India	Asian	White blood cells	Yes
Ueda and colleagues[67]	144/54	Non-epidemiological study	2000–04	Japan	Asian	Exfoliated cervical cells	Yes
2006							
Ciotti and colleagues[68]	30/32	Non-epidemiological study	2002–05	Italy	White	Exfoliated cervical cells	Yes
Gudleviciene and colleagues[69]	141/79	Non-epidemiological study	2000–03	Lithuania	White	Exfoliated cervical cells	No
Ueda and colleagues[70]	79/95*	Non-epidemiological study	2003–05	Japan	Asian	White blood cells	No
Unpublished							
Schmitt and colleagues	62/64	Epidemiological study (case-control)	1999–2001	Brazil	Mixed	Exfoliated cervical cells	Yes

NA=not applicable. NK=not known. *Controls were not cytological or histological confirmed and therefore excluded from analysis dataset. †Controls were not included in original data received. ‡Controls were newborn babies and therefore completely excluded from pooled reanalysis.

Table 1: **Overview of the 49 studies included in the pooled reanalysis with individual data**

established in several studies, and others selectively tested for just a few selected HPV types, such as HPV16.

Individual risk estimates were calculated and presented as forest plots by study type for all 25 studies included in the analysis (figure 2), and for five epidemiological studies with controls in Hardy–Weinberg equilibrium and *TP53* genotype determined from white blood cells (figure 3).

The pooled estimates across all studies showed an association between *TP53* codon 72 polymorphism for arginine homozygosity and invasive (1·22; 95% CI 1·08–1·39) or squamous cervical cancer (1·19; 1·03–1·36) compared with heterozygosity (table 4). No association was shown for arginine homozygosity versus proline homozygosity for invasive (1·13; 0·94–1·35) or squamous cervical cancer (1·17; 0·95–1·43). However, the overall genotype effect was statistically significant for invasive and squamous cancer. The pooled estimates for arginine homozygosity versus heterozygosity plus proline homozygosity as the reference and adjusted for each study showed statistically significant increased OR for invasive cervical cancer (1·20; 1·07–1·35) and squamous-cell carcinoma (1·18; 1·04–1·35; table 4).

See Online for webappendix

We analysed subgroups of all patients with invasive cancer and available controls for HPV status and ethnic group (table 4). A statistically significant increased risk was found for white women. No excess risk was found for the subgroups of Asian and Hispanic women.

Analysis of subgroups of patients with invasive cancer on the basis of quality criteria showed that the increased risks were found in studies in which the controls did not show Hardy–Weinberg equilibrium (compared with those that did, 1·71; 95% CI 1·21–2·42), in studies that were not sound epidemiologically (compared with those that were, 1·35; 1·15–1·58), and studies in which the *TP53* codon 72 genotype was determined by use of tumour tissue from cases (compared with white blood cells, 1·39; 1·13–1·73; table 4). Epidemiological studies and studies in which *TP53* polymorphism was determined from white blood cell DNA showed no association in any subgroup analyses.

Additionally, we restricted our analysis to a dataset of six studies with 705 cases and 933 controls derived solely from epidemiological studies with controls in Hardy–Weinberg equilibrium and *TP53* genotype detection from white blood cells (table 5). No increased risks were seen in any of the groups for invasive cervical cancer nor for squamous-cell carcinoma.

Further, we did a sensitivity analysis based on all studies where *TP53* polymorphism was determined from white blood cells (table 6). In this analysis, one non-epidemiological study was included in addition to the epidemiological studies (webappendix). No increased risk estimates were found in any subgroup.

We also did all analyses with a complete dataset of 2139 cases and 4607 controls from 17 studies with no missing values for age, HPV infection, or ethnic group (webappendix). By contrast with the results shown in table 4, we found no significant increased risk for either invasive cancer or squamous-cell carcinoma.

An analysis in which the two alleles were considered separately (n=13 227) and the proline alleles were used as the reference group showed similar results when analysis was restricted to epidemiological studies with controls in Hardy–Weinberg equilibrium and *TP53* genotype detection from white blood cells (webappendix), or was restricted to studies where *TP53* genotype was determined from white blood cells.

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	n
Publication year	
1998–2001	26
2002–06	22
Unpublished	1
Method of *TP53* genotyping	
Allele-specific PCR	23
RFLP (partly combined with allele-specific PCR)	19
SSCP	7
Material used for *TP53* genotyping	
White blood cells	13
Exfoliated cervical cells	13
Tumour tissue (cases)	17
Mixed (tumour tissue for some cases)	6
Method of HPV detection	
MY9/11 PCR	17
GP5+/6+ PCR	4
E6/E7 PCR	5
L1-related PCR	1
Hybrid capture 2	2
More than one	7
Others	3
Unknown	4
No HPV detection performed	6
Method of HPV genotyping	
Allele-specific PCR	19
Sequencing	3
RFLP	6
SSCP	1
Antibodies	2
More than one	3
Unknown	7
No genotyping performed	8
Hardy-Weinberg equilibrium (controls)	
Yes	27
No	6
No control group*	16
Study size†	
≥200 participants	25
<200 participants	24
Study type	
Epidemiological study	16
Non-epidemiological	33

HPV=human papillomavirus. RFLP=restriction fragment length polymorphism. SSCP=single-strand conformation polymorphism. *Three case series and 13 studies from which all controls were excluded because their control status was not confirmed by cytology or histology. †Total study size.

Table 2: **Characteristics of 49 included studies comprising 15 834 women**

	Cases: n (%)	Controls: n (%)
Histology and cytology		
Squamous cervical cancer	2763 (34·8)	..
Adenocarcinoma or adenosquamous carcinoma	311 (3·9)	..
Cervical cancer of unknown type	623 (7·8)	..
High-grade lesions*	2587 (32·6)	..
Low-grade lesions†	1487 (18·7)	..
Lesions (grade unclear)	175 (2·2)	..
Negative‡	..	7888 (100)
Age (years)		
15–29	804 (10·1)	2377 (30·1)
30–49	2326 (29·3)	2627 (33·3)
50–69	1074 (13·5)	813 (10·3)
≥70	155 (2·0)	60 (0·8)
No data	3587 (45·1)	2011 (25·5)
***TP53* genotype**		
Arginine homozygote	3562 (44·8)	3613 (45·8)
Arginine or proline heterozygote	3349 (42·2)	3431 (43·5)
Proline homozygote	1035 (13·0)	844 (10·7)
Ethnic group		
White	3517 (44·3)	3063 (38·8)
Asian	2704 (34·0)	2067 (26·2)
Black	311 (3·9)	54 (0·7)
Hispanic	754 (9·5)	2575 (32·6)
Mixed	210 (2·6)	64 (0·8)
No data	450 (5·7)	65 (0·8)
HPV status		
High-risk positive	4478 (56·4)	1060 (13·4)
Positive for other types or type unknown	617 (7·8)	293 (3·7)
Negative	936 (11·8)	5096 (64·6)
No data	1915 (24·1)	1439 (18·2)
Number of HPV types in HPV-positive women (n=6448)		
1	4026 (79·0)	928 (68·6)
≥2	584 (11·5)	293 (21·7)
No data	485 (9·5)	132 (9·8)

*High-grade squamous intraepithelial lesions and cervical intraepithelial lesions grades 2 and 3. †Low-grade squamous intraepithelial lesions and cervical intraepithelial lesions grade 1. ‡Confirmed by cytology or histology.

Table 3: **Characteristics of 7946 cases and 7888 controls included in the analysis**

Discussion

Our pooled analysis of original individual data from 49 studies provides evidence that there is no association between cervical cancer and *TP53* codon 72 polymorphism when the analysis is restricted to methodologically sound studies, and shows that the excess risks reported in several studies are due to methodological errors such as selection bias, the material used for genotype determination, or chance.

Combining data from many studies has the advantage of reducing random error. Often, small studies of genetic associations have insufficient power, increasing the risk that chance could be responsible for their conclusions.[71] Pooled analysis enabled us to apply the same kind of criteria to all the study datasets and to obtain precise estimates for subgroups. However, although all data were pooled from separate studies into a common database, each study was accounted for in the statistical models to allow for the widely varying proportions of cases and controls, and to avoid implicitly comparing cases of one study with controls of another. Additionally, each ethnic

	Arg/Arg		Arg/Pro+Pro/Pro		Odds ratio (95% CI)
	Cases	Total	Cases	Total	
Cohort studies					
Hildesheim and colleagues (Costa Rica) 1998	12	90	18	145	1·09 (0·50–2·37)
Hildesheim and colleagues (Portland, USA) 1998	5	120	1	69	2·96 (0·34–25·84)
Case-control studies					
Bhattacharya and colleagues 2002	32	87	61	207	1·39 (0·82–2·36)
Helland and colleagues 1998	44	166	33	136	1·13 (0·67–1·90)
Josefsson and colleagues 1998	34	287	29	269	1·11 (0·66–1·88)
Kim and colleagues 2001	40	92	60	108	0·62 (0·35–1·08)
Klug and colleagues 2001	57	121	58	117	0·91 (0·54–1·51)
Schmitt and colleagues	13	42	9	44	1·74 (0·65–4·65)
Settheetham-Ishida 2004	26	46	85	179	1·44 (0·75–2·76)
Tachezy and colleagues 1999	34	74	33	66	0·85 (0·44–1·65)
Tenti and colleagues 2000	59	146	42	95	0·86 (0·51–1·44)
Non-epidemiological studies					
Abba and colleagues 2003	12	42	23	72	0·85 (0·37–1·96)
Agorastos and colleagues 2000	8	14	4	28	8·00 (1·79–35·74)
Giannoudis and colleagues 1999	27	44	16	29	1·29 (0·50–3·34)
Gudleviciene and colleagues 2006	33	43	88	157	2·59 (1·19–5·61)
Jee and colleagues 2003	159	453	213	643	1·09 (0·85–1·41)
Kawamata and colleagues 2002	21	64	23	92	1·47 (0·72–2·96)
Klaes and colleagues 1999	33	119	25	95	1·07 (0·58–1·97)
Nagpal and colleagues 2002	21	24	50	76	3·64 (0·99–13·35)
Pillai and colleagues 2002	36	55	147	238	1·17 (0·63–2·17)
Saranath and colleagues 2002	27	46	103	215	1·55 (0·81–2·95)
Suárez-Rincón and colleagues 2002	17	33	21	57	1·82 (0·76–4·34)
Van Duin and colleagues 2000	44	93	27	64	1·23 (0·65–2·34)
Yamashita and colleagues 1999	28	108	48	220	1·25 (0·73–2·14)
Zehbe and colleagues 2001 Sweden	50	138	29	129	1·96 (1·14–3·36)

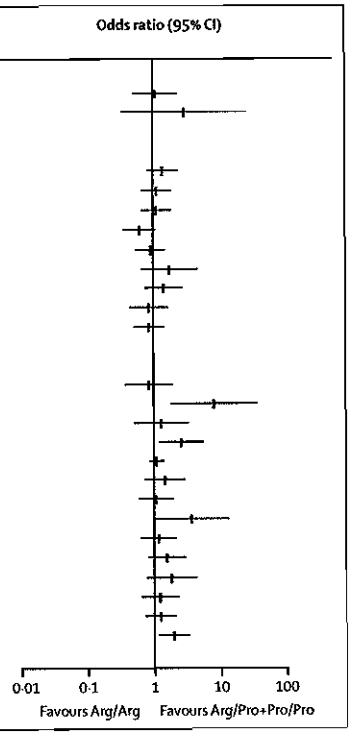

Figure 2: **Forest plots of effect estimates for patients with invasive cancer and cytology-negative controls of 25 individual studies stratified by type of study, only considering studies with at least five cases and five controls**

	Arg/Arg		Arg/Pro+Pro/Pro		Odds ratio (95% CI)
	Cases	Total	Cases	Total	
Cohort studies					
Hildesheim and colleagues (Costa Rica) 1998	12	90	18	145	1·09 (0·50–2·37)
Case-control studies					
Helland and colleagues 1998	44	166	33	136	1·13 (0·67–1·90)
Klug and colleagues 2001	57	121	58	117	0·91 (0·54–1·51)
Settheetham-Ishida 2004	26	46	85	179	1·44 (0·75–2·76)
Tachezy and colleagues 1999	34	74	33	66	0·85 (0·44–1·65)

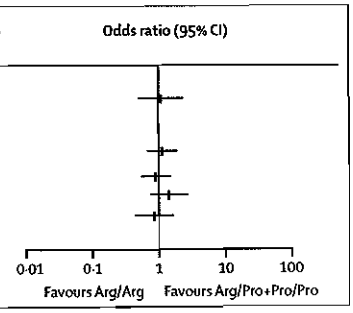

Figure 3: **Forest plots of effect estimates for invasive cancer cases and cytological negative controls of five individual epidemiological studies where controls were in Hardy-Weinberg equilibrium and *TP53* genotype was determined from white blood cells**
Stratified by type of study, only considering studies with at least five cases and five controls.

group was considered separately, either because a study only included one ethnic group, or by considering different ethnic groups within one study as separate studies.

No statistical heterogeneity of the genotype effect was detected between studies; however, the power of this test is low. The included studies had various designs and methodology, with some following epidemiological principles while others were laboratory based. Other differences included the definition of the study population, the recruitment of participants to the study, the information available on study participants, and the materials and methods used to determine *TP53* genotype and HPV status. We addressed variability between studies by using subgroups focusing on the biological and epidemiological contents rather than solely on statistical heterogeneity. Our subgroup analyses were planned a priori, but nevertheless we consider them to be explorative.

Pooling of the original data enabled us to redefine the genotype classifications and reference groups in the statistical analyses.[32] The main analysis was done with all three genotypes as one variable with three separate categories. This avoids the inflation of type I error that can occur with post-hoc grouping of genotypes, and enables estimation of all contrasts between ungrouped genotypes.[32] Additionally, the genotypes were grouped into a dichotomous variable,

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	Cases/controls*	Number of studies*	TP53 genotype as variable with three categories (Arg/Arg, Arg/Pro, Pro/Pro), different comparisons					TP53 genotype as combined variable with two categories	
			Arg/Arg vs Arg/Pro (reference): odds ratio adjusted for study (95% CI)†	Arg/Arg vs Pro/Pro (reference): odds ratio adjusted for study (95% CI)†	Arg/Pro vs Pro/Pro (reference): odds ratio adjusted for study (95% CI)†	p value‡	p value for interaction§	Arg/Arg vs Pro/Pro and Arg/Pro (reference): odds ratio adjusted for study (95% CI)†	p value¶
Cervical histology									
Invasive cervical cancer[18,19,22–25,35,39,40,43,47–49,52,54–56,63,69]‖**	2118/3979	25	1·22 (1·08–1·39)	1·13 (0·94–1·35)	0·92 (0·77–1·10)	0·01/0·12	..	1·20 (1·07–1·35)	0·14
Squamous cell carcinoma[19,22,23,25,35,39,43,47–49,52,54,55,57,59,60,63,69]	1600/3179	18	1·19 (1·03–1·36)	1·17 (0·95–1·43)	0·98 (0·80–1·20)	0·05/0·12	..	1·18 (1·04–1·35)	0·16
Adenocarcinoma and adenosquamous carcinoma[22,35,47,54,60,69]	145/1391	6	1·11 (0·76–1·64)	0·90 (0·50–1·60)	0·81 (0·45–1·44)	0·73/0·28	..	1·06 (0·74–1·53)	0·31
High-grade lesions[18,19,24,25,34,36,38,39,43,49,50,53–56,59,60,64,65,67,68]‖**††	2106/6614	23	1·05 (0·93–1·18)	1·05 (0·87–1·27)	1·00 (0·83–1·21)	0·71/0·31	..	1·05 (0·94–1·18)	0·15
Low-grade lesions[18,19,24,34,38,39,43,50,53,55,56,59,60,65,67,68]** ‡‡	1281/5239	17	1·08 (0·93–1·27)	0·86 (0·67–1·08)	0·79 (0·62–1·00)	0·14/0·91	..	1·03 (0·89–1·20)	0·64
HPV status§§									
Positive[22–24,35,40,48,52,55,56,59,60,63,69]‖**	953/522	15	1·30 (0·99–1·71)	0·91 (0·61–1·37)	0·70 (0·47–1·05)	0·08/0·36	..	1·20 (0·93–1·56)	0·55
High-risk positive[22–24,35,40,48,52,55,56,59,60,63,69]‖**	912/435	15	1·34 (1·00–1·80)	0·90 (0·58–1·41)	0·67 (0·44–1·04)	0·07/0·31	..	1·23 (0·93–1·64)	0·53
Unknown[58,60]	384/718	2	1·12 (0·86–1·46)	1·41 (0·94–2·13)	1·26 (0·84–1·90)	0·24/0·55	..	1·18 (0·91–1·51)	0·30
Ethnic group§§									
White[18,19,22–25,35,39,40,47,48,69]	704/1695	12	1·34 (1·09–1·65)	1·04 (0·73–1·48)	0·77 (0·54–1·11)	ND/0·22	0·64	1·28 (1·05–1·56)	0·10
Asian[43,49,52,54–57,59,63]	1180/1773	9	1·19 (1·00–1·42)	1·14 (0·90–1·43)	0·95 (0·77–1·18)	ND/0·07		1·18 (1·00–1·39)	0·23
Hispanic[22,24,58,59]	212/447	4	1·01 (0·69–1·46)	1·10 (0·60–2·00)	1·09 (0·60–1·99)	ND/0·52		1·02 (0·72–1·46)	0·51
Quality criteria§§									
Controls in Hardy-Weinberg equilibrium[18,19,22–24,36,39,40,43,47–49,52,54–56,59,60,63]‖**	1766/3224	21	1·16 (1·01–1·32)	1·16 (0·95–1·42)	1·00 (0·83–1·22)	¶¶/0·25	0·04¶¶	1·16 (1·02–1·32)	0·17
Controls not in Hardy-Weinberg equilibrium[25,57,58,69]	352/755	4	1·71 (1·21–2·42)	1·06 (0·67–1·69)	0·62 (0·41–0·95)	¶¶/0·24		1·54 (1·11–2·15)	0·34
Epidemiological study[22–25,35,42,49,52,63]‖**	785/1921	11	1·06 (0·87–1·29)	0·94 (0·71–1·26)	0·89 (0·67–1·18)	ND/0·42	0·11	1·03 (0·86–1·25)	0·54
Non-epidemiological study[18,19,39,40,43,48,54–60,69]	1333/2058	14	1·35 (1·15–1·58)	1·28 (1·01–1·62)	0·95 (0·76–1·18)	ND/0·13		1·34 (1·14–1·56)	0·14
≥200 participants[19,22,24,25,36,40,43,47,49,52,55,57,60,63,69]‖	1715/3424	16	1·19 (1·04–1·37)	1·06 (0·88–1·29)	0·89 (0·74–1·08)	ND/0·07	0·30	1·16 (1·02–1·33)	0·27
<200 participants[18,23,39,48,54,55,58,59]**	403/555	9	1·36 (1·02–1·82)	1·61 (0·97–2·66)	1·18 (0·72–1·94)	ND/0·57		1·40 (1·06–1·84)	0·14
TP53 genotype determined from white blood cells[22–24,35,60,69]	772/1464	6	1·06 (0·87–1·29)	1·11 (0·84–1·48)	1·05 (0·79–1·39)	ND/0·39	0·32	1·07 (0·89–1·29)	0·89
TP53 genotype of cases determined from tumour tissue[18,19,39,43,47,48,52,54,55,58,59]	663/1199	11	1·39 (1·13–1·73)	1·38 (0·97–1·96)	0·99 (0·70–1·40)	ND/0·20		1·39 (1·14–1·71)	0·14
TP53 genotype determined from exfoliated cells[24,25,60,69]**	270/975	5	1·45 (1·03–2·04)	1·23 (0·73–2·08)	0·85 (0·50–1·43)	ND/0·49		1·40 (1·02–1·94)	0·33

ND=not determined. *Numbers refer to number of studies or number of cases and controls contributing to each subgroup. †In these logistic regression models, only studies that contributed more than four cases and more than four controls per subgroup and ethnic group were included, participants with unknown ethnic group were excluded. ‡p value of effect and p value of heterogeneity between studies. §p value of heterogeneity between subgroups. ¶p value of heterogeneity between studies. ‖Publication of Hildesheim and colleagues contributed two studies. **Unpublished data of Schmitt. ††Confirmed by histology or cytology; includes high-grade squamous and cervical intraepithelial lesions grades 2 and 3. ‡‡Confirmed by histology or cytology; includes low-grade squamous and cervical intraepithelial lesions grade 1. §§For invasive cervical cancer cases and controls only. ¶¶p value of effect was significant for controls not in Hardy-Weinberg equilibrium but not for controls in Hardy-Weinberg equilibrium after Bonferroni adjustment at the nominal level of 0·025.

Table 4: **Pooled estimates of associations of *TP53* codon 72 genotype and cervical histology of 33 studies, and invasive cervical cancer cases of 25 studies in subgroups of HPV status, ethnic group, and different quality criteria**

in part for direct comparison with an earlier meta-analysis with published data.[27]

Invasive cancer cases and high-grade and low-grade lesions were analysed separately. Although some effects were found overall with invasive and cervical cancer, analyses of high-grade and low-grade lesions showed no increased risks. Furthermore, the genotype effect was seemingly not modified by HPV infection status. As the distribution of alleles at codon 72 of the *TP53* gene varies by ethnic group,[33,71] we analysed data stratified by ethnicity. Although the risk estimates varied by ethnic group, none was significant in analyses of methodologically sound studies (table 5).

To assess study quality, we considered Hardy–Weinberg equilibrium, study type, study size, and the material used for *TP53* genotyping in subgroup analyses. The concern was that any association detected might be spurious if the distribution of genotypes in the control groups were not

	Cases/controls*	Number of studies*	TP53 genotype as variable with three categories (Arg/Arg, Arg/Pro, Pro/Pro), different comparisons				TP53 genotype as combined variable with two categories	
			Arg/Arg vs Arg/Pro (reference): OR adjusted for study (95% CI)†	Arg/Arg vs Pro/Pro (reference): OR adjusted for study (95% CI)†	Arg/Pro vs Pro/Pro (reference): OR adjusted for study (95% CI)†	p value‡	Arg/Arg vs Pro/Pro and Arg/Pro (reference): OR adjusted for study (95% CI)†	p value§
Cervical histology								
Invasive cervical cancer[22-24,35,63]	400/740	5	1·07 (0·81–1·42)	0·98 (0·65–1·48)	0·92 (0·61–1·37)	0·86/0·31	1·05 (0·80–1·37)	0·79
Squamous cell carcinoma[22,23,35,63]	331/535	4	1·01 (0·74–1·39)	1·11 (0·70–1·76)	1·10 (0·71–1·71)	0·90/0·49	1·03 (0·77–1·39)	0·49
Adenocarcinoma and adenosquamous carcinoma[22,35]	21/336	2	1·20 (0·46–3·13)	0·96 (0·20–4·57)	0·80 (0·16–4·12)	0·93/0·12	1·15 (0·47–2·79)	0·65
High-grade lesions[24,35,64]¶	305/623	3	1·01 (0·75–1·35)	1·06 (0·66–1·70)	1·05 (0·66–1·67)	0·97/0·38	1·02 (0·77–1·35)	0·37
HPV status‖								
Positive[22-24,35]**	273/176	4	1·37 (0·83–2·26)	1·19 (0·56–2·53)	0·87 (0·41–1·86)	0·47/0·14	1·32 (0·83–2·12)	0·88
High-risk positive[22-24,35]**	264/134	4	1·45 (0·84–2·52)	1·38 (0·59–3·20)	0·95 (0·41–2·20)	0·39/0·13	1·44 (0·85–2·42)	0·67
Ethnic group‖								
White[22,23,35]	150/310	3	1·13 (0·73–1·73)	0·69 (0·34–1·41)	0·61 (0·29–1·28)	0·43/0·26	1·03 (0·69–1·53)	0·77
Asian[63]	111/114	1	1·36 (0·69–2·69)	1·64 (0·74–3·65)	1·20 (0·63–2·30)	0·47/··	1·44 (0·75–2·76)	··
Hispanic[24]	30/205	1	1·25 (0·54–2·90)	0·65 (0·20–2·04)	0·52 (0·17–1·60)	0·51/··	1·09 (0·50–2·37)	··

*Numbers refer to number of studies or number of cases and controls contributing to each subgroup. †In these logistic regression models, only studies that contributed more than four cases and more than four controls per subgroup and ethnic group were included, participants with unknown ethnic group excluded. ‡p value of effect and p value of heterogeneity between studies. §p value of heterogeneity between studies. ¶Confirmed by histology or cytology; includes high-grade squamous and cervical intraepithelial lesions grades 2 and 3. ‖For invasive cervical cancer cases and controls only. **Publication of Hildesheim and colleagues contributed two studies.

Table 5: **Pooled estimates of associations of *TP53* codon 72 genotype and cervical disease of six studies; and invasive cervical cancer cases of five studies, only studies with controls in Hardy–Weinberg equilibrium, epidemiological study, and genotyping with white blood cells**

	Cases/controls*	Number of studies*	*TP53* genotype as variable with three categories (Arg/Arg, Arg/Pro, Pro/Pro), different comparisons				*TP53* genotype as combined variable with two categories	
			Arg/Arg vs Arg/Pro (reference): odds ratio adjusted for study (95% CI)†	Arg/Arg vs Pro/Pro (reference): odds ratio adjusted for study (95% CI)†	Arg/Pro vs Pro/Pro (reference): odds ratio adjusted for study (95% CI)†	p value‡	Arg/Arg vs Pro/Pro and Arg/Pro (reference): odds ratio adjusted for study (95% CI)†	p value§
Cervical histology								
Invasive cervical cancer[22-24,35,60,63]	772/1464	6	1·06 (0·87–1·29)	1·11 (0·84–1·48)	1·05 (0·79–1·39)	0·72/0·39	1·07 (0·89–1·29)	0·89
Squamous cell carcinoma[22,23,35,60,63]	647/1259	5	1·02 (0·83–1·26)	1·19 (0·87–1·62)	1·16 (0·85–1·57)	0·55/0·69	1·06 (0·87–1·29)	0·65
Adenocarcinoma and adenosquamous carcinoma[22,35,60]	74/1060	3	1·10 (0·66–1·82)	1·24 (0·56–2·75)	1·13 (0·51–2·52)	0·85/0·28	1·13 (0·70–1·82)	0·90
High-grade lesions[24,35,60,64]¶	589/1347	4	0·96 (0·78–1·18)	0·98 (0·72–1·35)	1·03 (0·75–1·40)	0·92/0·61	0·96 (0·79–1·17)	0·51
Low-grade lesions[66]‖	85/724	1	1·12 (0·68–1·83)	0·81 (0·42–1·54)	0·73 (0·38–1·38)	0·62/··	1·02 (0·65–1·62)	··
HPV status**								
Positive[22-24,35,60,63]††	396/258	6	1·45 (0·96–2·20)	0·90 (0·49–1·67)	0·62 (0·34–1·13)	0·12/0·08	1·31 (0·88–1·94)	0·48
High-risk positive[22-24,35,60,63]††	387/214	6	1·56 (1·00–2·44)	0·98 (0·50–1·91)	0·63 (0·33–1·20)	0·10/0·05	1·41 (0·92–2·17)	0·24
Unknown[69]	346/666	1	1·07 (0·81–1·41)	1·37 (0·90–2·09)	1·28 (0·84–1·95)	0·34/··	1·13 (0·87–1·47)	··
Ethnic group**								
White[22,23,35]	150/310	3	1·13 (0·73–1·73)	0·69 (0·34–1·41)	0·61 (0·29–1·28)	0·43/0·26	1·03 (0·69–1·53)	0·77
Asian[60,63]	483/838	2	1·09 (0·85–1·39)	1·30 (0·92–1·86)	1·20 (0·86–1·69)	0·34/0·76	1·13 (0·89–1·43)	0·44
Hispanic[22,24]	139/316	2	0·94 (0·59–1·51)	0·89 (0·44–1·78)	0·94 (0·46–1·91)	0·93/0·40	0·93 (0·60–1·44)	0·64

*Numbers refer to number of studies or number of cases and controls contributing to each subgroup. †In these logistic regression models, only studies that contributed more than four cases and more than four controls per subgroup and ethnic group were included, participants with unknown ethnic group were excluded. ‡p value of effect and p value of heterogeneity between studies. §p value of heterogeneity between studies. ¶Confirmed by histology or cytology; includes high-grade squamous and cervical intraepithelial lesions grades 2 and 3. ‖Confirmed by histology or cytology; includes low-grade squamous and cervical intraepithelial lesions grade 1. **For invasive cervical cancer cases and controls only. ††Publication of Hildesheim and colleagues contributed two studies.

Table 6: **Pooled estimates of associations of *TP53* codon 72 genotype and cervical disease of the seven studies that used white blood cells to determine *TP53* genotype**

in Hardy–Weinberg equilibrium.[74] It is essential to calculate Hardy–Weinberg equilibrium in meta-analyses of genetic polymorphisms in order to assess quality, genotyping errors, and selection bias.[32,75] We noted that studies where the controls were not in Hardy–Weinberg equilibrium showed statistically significantly increased

odds ratios compared with studies in which the controls were in Hardy–Weinberg equilibrium. We also classified the studies as being either epidemiological or non-epidemiological on the basis of differences in study design, definition and recruitment of study participants, and study conduct.[71] This was an attempt to assess error and bias, which sometimes skew the results of studies in molecular epidemiology,[76–78] with information available in all studies pooled in this analysis. Studies that adhered to sound epidemiological principles gave null results.

Loss of heterozygosity is a frequent event in tumour cells,[79] and it has been postulated that this frequently affects the proline allele in squamous cell carcinomas.[80] Additionally, loss of heterozygosity has been noted in exfoliated cells.[81] Therefore, DNA from white blood cells should be used for determining genetic polymorphisms rather than tumour tissue. Studies that met this criterion did not show an association between cervical cancer and the *TP53* polymorphism. Moreover, all studies considered as epidemiological studies and all studies where *TP53* genotype was detected from white blood cells and which contributed to the analyses shown had controls in Hardy–Weinberg equilibrium. By contrast, in the subgroups of studies using tumour tissue or exfoliated cells, only two of 11 studies or one of five studies were methodologically sound, respectively, while the rest were either not done according to epidemiological criteria, were small, or had controls not in Hardy–Weinberg equilibrium (or a combination of all three).

Recently, an interim guideline was published to assess cumulative evidence on genetic associations, such as the amount of biological evidence, epidemiological credibility, and clinical and public-health impact.[71] There is biological evidence that the two different *TP53* codon 72 protein variants could have differential effects on cancer development. Our pooled analysis adds large-scale epidemiological evidence with extensive replication. There is no statistically significant between-study heterogeneity; although there is moderate epidemiological heterogeneity between studies. The effects of potential selection of study populations or genotyping errors were assessed with subgroup analyses. There is evidence that the associations between *TP53* codon 72 polymorphism and cervical cancer noted in earlier studies were due to selection bias. Our pooled analysis gives moderate-to-strong epidemiological evidence that there is no association between *TP53* codon 72 polymorphism and cervical cancer.

The median number of individuals was 255 in the 49 included studies and 161 in 32 studies for which no data were provided (figure 1, table 1, webappendix). The proportion of studies that used white blood cells to determine *TP53* genotype was similar in studies included and not included in this analysis (26·5% *vs* 25·0%). For the studies from which original data were not available, we used a random-effects model based on published numbers to calculate the pooled-effect estimate[82] for all studies of invasive cervical cancer in which the absence of cervical disease in controls had been confirmed (n=10). The odds ratio for arginine homozygosity versus heterozygosity plus proline homozygosity was increased but not statistically significant (1·37; 95% CI 0·93–2·00). The original data of Storey and colleagues[17] which initiated this research were not included in this analysis because they were not received for pooling. Nevertheless, because it was a very small study (30 cases and 41 controls), the data would not have altered the results of this pooled analysis. It can be assumed that pooled analysis of the data from all 77 published articles would not change our conclusions. However, it can not be ruled out that the *TP53* polymorphism might have some role in determining age of onset, aggressiveness of disease, cancer survival, or mortality.[12,83,84]

Our study has a number of possible limitations, including the possibility that genotyping errors in the individual studies could have introduced bias at the pooled level.[71,85] Also, no data were available on risk factors such as smoking, use of oral contraceptives, or parity. Furthermore, even pooled analyses of original data are not exempt from publication bias;[86] however, many studies, both large and small, that gave null results on *TP53* polymorphism and cervical cancer have been published. Nevertheless, we attempted to identify and partly succeeded in including unpublished work. An investigation of publication bias showed that data from small studies were more likely to be published or received for this pooled analysis if there was an increased risk reported. Another potential limitation was the smaller sample size in subgroups than in the main analyses. In the main analysis of all invasive cancer cases the minimum odds ratio detectable with a power of 80% was 1·18 for arginine homozygotes versus heterozygotes. For the subgroups of studies that had sound epidemiological quality or that used white blood cells for genotyping, the minimum odds ratios detectable with a power of 80% were 1·27 or 1·30, respectively, for arginine homozygotes versus heterozygotes. Therefore, the power in subgroup analyses was not sufficient to detect small increased risks. However, such small increased risks would have no or minimal clinical or public-health effects.

After a decade of extensive research on this issue, this large pooled analysis found no association between cervical cancer and polymorphism at *TP53* codon 72 when analysis was restricted to methodologically sound studies. The increased risks of cervical cancer associated with the polymorphism at *TP53* codon 72 seen in some studies were probably due to errors in study methods, rather than to biological or clinical factors.

Contributors

SJK planned and initiated the project, contacted the collaborators, planned and discussed the statistical analysis, and wrote the paper. MR pooled the data, did the statistical analysis, and helped to write the paper. JK discussed and did the statistical analysis, and discussed the paper. MCA, TA, SMFB, MC, BRD, ADM, AD, ARG, ZG, UG, ALFH, AH, CSH, OH, SHJ, JWK, MMM, JM, HYSN, AN, YN, RJP, MRP, GNR, GR, ANR, SR, DS, VMS, SS, WS-I, HS, PJFS, MHS, AES-R, KS, RT, MU, AGJvdZ, MvKD, MTW, TY, and IZ collected and contributed original individual data, discussed the analysis, and discussed the paper. MB supported the project in all its phases, discussed the statistical analysis, and discussed the paper.

Conflict of interest
The authors declared no conflicts of interest.

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Genetic risk of *XRCC1* and *XRCC3* polymorphism for cervical cancer in Northeast Thai

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Abstract

Objective. Cervical cancer is still a serious national health problem in Thailand and risk factors other than high risk HPV infection for the cancer should be identified to reduce the new cases of the cancer. Since the influence of the polymorphisms of DNA repair genes on the development of cervical cancer was unknown, we have selected BER related XRCC1 and DBS related XRCC3 to test the contribution of their polymorphisms, XRCC1 Arg399Gln and Arg194Trp and XRCC3 Thr241Met, to develop cervical cancer.

Methods. Women aged 27-74 years with the HPV infection were recruited in this study. Cases (n=111) were defined as squamous cell cervical cancer and controls (n=118) were recruited from healthy women without cervical abnormalities.

Results. The *XRCC1* 194Trp/Trp genotype significantly increased the risk for cervical cancer (OR=5.52; 95%CI=1.14-26.64; p=0.03). Among the HPV infection negative group, significantly higher risks for SCCA were visualized for *XRCC1* 399Arg/Gln (adjusted OR=3.69; 95%CI=1.04-13.06; p=0.04) and *XRCC1* 194Arg/Trp (adjusted OR=4.13; 95%CI=1.13-15.12; p=0.03).

Conclusion. This study indicates that variant types of DNA repair genes play partial roles in modifying individual susceptibility to cervical cancer. Since cervical cancer is a

multi-factorial disease, the contribution of repair enzymes if it ever exists to the development of cervical caner is concealed by HPV infection.

Introduction

Cervical cancer is a serious national health problem in Thailand, especially northern and northeastern parts of the country [1]. To reduce the new cases of the cancer, risk factors for the cancer should be identified. Human papillomavirus (HPV) is a principal cause for cervical cancer, nearly 90 % of cervical cancer cases were with high-risk HPV infection in this region [2]. Although prophylactic vaccines against HPV have been developed, they are likely to be effective in preventing certain types of HPV (HPV16 and HPV18) infection, and about 30% of cervical cancers remain surrendered [3]. Cryptic risks for cervical cancer thus should be unveiled.

It is documented that the body's defense mechanism is able to eradicate HPV without progressing to the cervical cancer [4]. A small part of HPV carriers develop cervical cancer, which indicates the presence of some factor(s) responsible for the cervical cancer development other than HPV infection. Although HPV infection is the major risk for the development of cervical cancer, contribution of other risk factors is also indispensable. Our previous study has shown that passive tobacco smoking contributes to the increased risk for SCCA development in Northeast Thai women [5].

Carcinogens, such as nicotine, cotinine and tobacco-specific nitrosamines, have been detected in the cervical mucus of smokers [6-7]. Being able to induce DNA damages [8],they play causal roles in the development of cervical cancer [7]. On the other hand, DNA-repair systems are essential for the maintenance of integrity of the genetic material. Therefore they play a key role in protecting the genetic material against deleterious mutations leading to cancer development [9]. Among the DNA-repair systems, base-excision repair (BER) pathway and double-strand break (DSB) repair pathway constitute the primary defense against lesion generated by ionizing radiation and strong alkylating agents as well as lesions formed by endogenous DNA-damaging agents such as smoke [8] and viruses [10].

There has been considerable interest in understanding genetic variability in DNA repair genes and their influence on modifying an individual's susceptibility to cancer [11]. X-ray repair cross complementing group 1 (*XRCC1*) is a major DNA repair gene involved in BER, whereas XRCC3 is in DSB repair [9]. Mutation and polymorphism in DNA repair genes associated with repair efficiency against DNA damage may predispose an individual's cancer susceptibility. Functional genetic polymorphisms of the *XRCC1* are Arg399Gln in the exon 10 and Arg194Trp in the exon 6 and that of the *XRCC*3 is Thr241Met in the exon 7. Inter-individual differences in the DNA-repair efficiency appear to be genetically determined [12-13].

The *XRCC1* codon194 variant allele was associated with the risk for oral cancer [14], hepatocellular carcinoma [15], thyroid carcinoma [16], head and neck cancer [17], adenocarcinoma of the lung [18] and breast cancer [19]. The *XRCC1* codon339 Gln was

shown to be a risk for cervical cancer [20]. However, combination of the *XRCC1* variant alleles might vary risk for the individual cancer. The protective effect of combined genotypes codon 194 and 339 of *XRCC1* has been observed for bladder cancer [21] and hepatocellular carcinoma [15] but not in lung cancer [22,23], prostate cancer [24], and oral cancer [25]. The *XRCC3* codon 241 variant allele was involved in an increased level of DNA adducts [26] and significant risk for bladder cancer [27], oral cancer [14,25], and moreover, increased risk for the cancer might be related to smoking and drinking [14]. However, many of the observations are not consistent [28,29] and influence of the polymorphisms of *XRCC1* and *XRCC3* on DNA repair capacity is still unclear. We have selected BER related XRCC1 and DBS related XRCC3 to test the contribution of their polymorphisms to develop cervical cancer.

Materials and Methods

Subjects

Women aged 27-74 years were recruited in this study at Srinagarind Hospital, Khon Kaen University, Thailand. Cases (n=111) were defined as squamous cell cervical cancer (SCCA) by cytological, colposcopical and histological diagnosis. Controls (n=118) were recruited from healthy women without cervical cancer, history of conization, hysterectomy or diseases associated with known risk factors for cervical cancer. The controls and cases were matched within 5-year age group. Prior to this study, they were examined for HPV infection [2]. They were informed of the purpose and experimental procedures of this study and written informed consent was obtained. This study was approved by the Ethics Committee of Khon Kaen University.

Genotyping assays

DNA was extracted from peripheral blood cells by using NaI method [30] and used as template in polymerase chain reaction (PCR). Polymorphism in DNA repair genes using PCR-RFLP was performed with the primers in Table 1. The primers were designed to amplify the regions of DNA that contain the polymorphic sites of interest: *XRCC1* Arg399Gln in exon 10 (G-->A), *XRCC1* Arg194Trp in exon 6 (C-->T) and *XRCC*3 Thr241Met in exon 7 (C-->T). The PCR condition consisted basically of the following : i) activation of Taq polymerase at 95 °C for 9 min, ii) 40 cycles of denaturation at 94°C for 1 min; annealing at 58 °C for 1 min.; elongation at 72 °C for 1 min, and iii) extension at 72 °C for 5 min. The PCR products were electrophoresed on 2.5% agarose gel and visualized by ethidium bromide staining. Genetic polymorphism of *XRCC1* and *XRCC3* were studied by digesting the products with restriction enzymes as shown in Table 1.

Statistical analyses

The chi-square test was used to compare genotype frequency of the *XRCC1* (codon 194 and codon 399) and *XRCC3* (codon 241) polymorphism between the cervical carcinoma

patients and the controls. All subjects were pooled, cases and controls were matched 5-year age and then divided into small groups by combination with smoking or HPV status. The associations between *XRCC* genotypes and risk of cervical cancer with or without combination of smoking or HPV status were estimated using odds ratios and 95% confidence intervals (OR and 95% CI) calculated by multivariate logistic regression analysis with 800-STATA-PC. P-value less than 0.05 were considered significantly different.

Results

The allele frequencies and distribution of genotypes of the *XRCC1* codon 399 and 194, and *XRCC3* codon 241 are shown in Table 2. No significant deviation from Hardy-Weinberg equilibrium in the genotype distribution for the three loci was confirmed in the controls. The prevalence of the *XRCC1* 194Trp allele (T) was not significantly different in cases and controls ($p>0.05$) but *XRCC1* 194Trp/Trp genotype significantly increased the risk for cervical cancer (OR=5.52; 95%CI=1.14-26.64; p=0.03), whereas heterozygous genotype did not (OR=1.18; 95%CI=0.69-2.01; p=0.54). *XRCC1* 399 and *XRCC3* 241 polymorphisms did not alter the risk for the development of cervical cancer when we analyzed by genotype and allele distribution (Table 2). When ORs were calculated for combined genotypes of XRCC1 399 and 194, there was a trend to increase the risk for the cancer in Arg/Arg-Trp/Trp (G/G-T/T) genotype (OR=4.31; 95%CI=0.82-22.53; p=0.08) (Table 3). When genotypes were combined for three loci, the trend of increased risk in the presence of XRCC1 194Trp/Trp genotype was still observed (OR=4.08; 95%CI=0.77-21.54; p=0.09) (Table 4).

Interaction between *XRCC* genotypes and the risk for SCCA by the status of HPV infection was analyzed (Table 5). Among the HPV infection negative group, significantly higher risks for SCCA were visualized for *XRCC1* 399Arg/Gln (adjusted OR=3.69; 95%CI=1.04-13.06; p=0.04) and *XRCC1* 194Arg/Trp (adjusted OR=4.13; 95%CI=1.13-15.12; p=0.03). Other genotypes with the *XRCC1* 339Glu allele or the *XRCC1* 194Trp allele consistently showed higher risks even though the p values were not less than 0.05.

When risk of the XRCC polymorphisms for SCCA was evaluated by the smoking status, none of the genotypes showed deviation in the risk for the cancer statistically (Table 6).

Discussion

DNA repair is an important mechanism in the maintenance of genetic stability against carcinogenesis. Genetic variability in DNA repair genes is considerable and able to modify an individual's susceptibility to cancer [22-24]. Among the three nonsense polymorphic variants, only XRCC1 194 Trp/Trp showed an increased risk for SCCA (5.5-fold). The similar finding for the XRCC1 polymorphism in SCCA development was observed in Chinese [31]. This report [31] also observed XRCC1 399 Gln showed higher risks for the cervical cancer. On the contrary, Niwa et al. reported that *XRCC1* 399Gln was not the risk for SCCA but the risk for cervical adenocarcimona/adenosquamous carcinoma [20]. We often face such discrepancies among reports based on the different populations with different environmental backgrounds and moreover, not equally adjusted ORs would give higher risks to certain genotypes. Combined genotypes for XRCC1 399 and 194 showing trends in the increased risk also comprised the XRCC1 194 Trp/Trp homozygous genotype; this indicates functional compensation of XRCC1 194 Arg being strong. As for the XRCC3, relatively low (-0.05) allele frequency of XRCC3 241 Met allele limited the statistical analysis. To evaluate the effect of Met allele on SCCA, a further study with a large number of subjects is required.

Among SCCA cases, 13.5 % were found to be negative for HPV DNA in this study. When subjects were sorted by high risk HPV infection status, a clear trend was visualized (Table 5); contribution of XRCC1 variant alleles to the risk for SCCA was identified in the HPV negative group. In the HPV infection group, there was a trend that rather low risks for CC were found in the presence of the XRCC1 variant alleles. Increased risk for SCCA was found in both heterozygous genotypes, XRCC1 399 Arg/Gln and 194 Arg/Trp, among HPV negative status with the OR of 3.7 and 4.1-fold, respectively The contribution of XRCC1 399 Gln was thus unveiled by excluding influence of the established risk factor, high risk HPV infection. Our study indicates that among HPV carriers, strong driving force of HPV infection leads to the development of SCCA irrespective of XRCC genotype.

Our previous study showed smoking, including passive smoking, is one of the critical risks for the development of cervical carcinoma [5]. This means that some of the pro-carcinogens/carcinogens in tobacco smoke may be responsible for this cancer; however, the null genotype of phase I detoxification enzymes, GSTM1 and GSTT1, did not increase the risk for SCCA in smokers [32] and moreover, in this study, the variant allele for DNA repair proteins, XRCC1 and XRCC3, do not increase the risk. It is implicated that modification or activation of pro-carcinogens/carcinogens by metabolizing enzymes may play critical roles in the development of cervical cancer. To reveal the role of phase II enzymes, such as CYP1 and CYP2 families, in the development of cervical cancer is strongly recommended.

Totally, this study indicates that variant types of DNA repair genes play partial roles in modifying individual susceptibility to cervical cancer. Since cervical cancer is a multi-factorial disease, the contribution of repair enzymes if it ever exists to the development of the cervical cancer is concealed by the major risk factor, HPV infection, otherwise the increased risk should be found not only among HPV negative individuals but also HPV positive individuals.

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Table 1. PCR-RFLP assays for detecting polymorphisms in *XRCC1* and *XRCC3*

Polymorphism primer sequence (5'–3')	size of PCR products	RE	Genotype and size of RF products (bp)
XRCC1 codon 399			
Upper: caagtacagccaggtcctag	248	*Nci* I	Arg/Arg (G/G): 159, 89
Lower: ccttccctcatctggagtac			Arg/Gln (G/A): 248, 159, 89
			Gln/Gln (A/A): 248
XRCC1 codon194			
Upper: gccagggccctccttcaa	485	*Pvu* II	Arg/Arg (C/C): 485
Lower: taccctcagacccacgagt			Arg/Trp (C/T): 485, 396, 89
			Trp/Trp (T/T): 396, 89
XRCC3 codon 241			
Upper: ggtcgagtgacagtccaaac	455	*Nla* III	Thr/Thr (C/C): 315, 140
Lower: tgcaacggctgagggtctt			Thr/Met (C/T): 315, 210, 140, 105
			Met/Met (T/T): 210, 140, 105

Table 2 Risk of XRCC genotypes for SCCA

Genotype and frequency of variant allele	Number of subjects Cases	Controls	OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
XRCC1 codon 399				
Arg/Arg	66	69	1	1
Arg/Gln	41	44	0.97 [0.56-1.67], 0.95	1.45 [0.66- 3.18], 0.34
Gln/Gln	4	5	0.84 [0.21-3.25], 0.79	2.41 [0.36-16.05], 0.36
Arg/Gln+Gln/Gln	45	49	0.96 [0.57-1.63], 0.88	1.47 [0.69- 3.37], 0.31
freq. allele Gln	0.221	0.229 (p>0.05)		
XRCC1 codon 194				
Arg/Arg	53	65	1	1
Arg/Trp	49	51	1.18 [0.69- 2.01], 0.54	1.21 [0.57- 2.59], 0.61
Trp/Trp	9	2	5.52 [1.14-26.64], 0.03	6.73 [0.92-48.78], 0.06
Arg/Trp+Trp/Trp	58	53	1.34 [0.79- 2.25], 0.27	1.38 [0.67- 2.87], 0.38
freq. allele Trp	0.302	0.233 (p>0.05)		
XRCC3 codon 241				
Thr/Thr	101	106	1	1
Thr/Met	10	12	0.87 [0.36- 2.11],0.76	2.13 [0.61- 7.43], 0.23
freq. allele Met	0.045	0.051 (p>0.05)		

OR [95% CI]: Odds ratios [95% confidence interval]

*adjusted with multiple logistic regression for age, HPV status and smoking

Table 3 Risk of combined two XRCC1 polymorphisms for SCCA

XRCC genotype		Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
399	194	Cases	Controls		
Arg/Arg	Arg/Arg	26	32	1	1
Arg/Arg	Arg/Trp	33	35	1.16 [0.57- 2.34], 0.68	0.81 [0.30- 2.17], 0.68
Arg/Arg	Trp/Trp	7	2	4.31 [0.82-22.53], 0.08	2.93 [0.34-25.14], 0.32
Arg/Gln	Arg/Arg	24	28	1.05 [0.49- 2.24], 0.89	0.92 [0.32- 2.63], 0.88
Gln/Gln	Arg/Arg	3	5	0.74 [0.16- 3.38], 0.69	1.20 [0.14-10.52], 0.87
Arg/Gln	Arg/Trp	16	16	1.23 [0.52- 2.92], 0.64	1.96 [0.57- 6.73], 0.28

OR [95% CI]: Odds ratios [95%confidence interval]

*adjusted with multiple logistic regression for age, HPV status and smoking

Table 4 Combination of XRCCs genotypes and risk for SCCA

Genotype			Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
XRCC1		*XRCC3*				
399	194	241	Cases	Controls		
Arg/Arg	Arg/Arg	Thr/Thr	24	28	1	1
Arg/Arg	Arg/Arg	Thr/Met	2	4	0.58 [0.09- 3.47], 0.55	1.00 [0.08-11.71], 0.99
Arg/Arg	Arg/Trp	Thr/Thr	31	31	1.16 [0.56- 2.44], 0.68	0.78 [0.28- 2,20], 0.65
Arg/Arg	Arg/Trp	Thr/Met	2	4	0.58 [0.09- 3.46], 0.55	0.94 [0.07-12.83], 0.97
Arg/Arg	Trp/Trp	Thr/Thr	7	2	4.08 [0.77-21.54], 0.09	0.72 [0.31-23.74], 0.37
Arg/Gln	Arg/Arg	Thr/Thr	20	24	0.97 [0.43- 2.17], 0.94	0.82 [0.26- 2.52], 0.73
Arg/Gln	Arg/Arg	Thr/Met	4	4	1.17 [0.26- 5.17], 0.84	2.41 [0.32-18.22], 0.39
Arg/Gln	Arg/Trp	Thr/Thr	14	16	1.02 [0.41- 2.51], 0.96	1.51 [0.42- 5.48], 0.52
Gln/Gln	Arg/Arg	Thr/Thr	3	5	0.70 [0.15- 3.23], 0.65	1.10 [0.12- 9.69], 0.25

OR [95% CI]: Odds ratios [95%confidence interval]

*adjusted with multiple logistic regression for age, HPV status and smoking

Table 5 XRCC genotype, HPV status and risk for SCCA

Genotype	Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
	Cases	Controls		
HPV negative				
XRCC1 codon 399				
Arg/Arg	5	56	1	1
Arg/Gln	9	35	2.88 [0.89- 9.29], 0.08	3.69 [1.04-13.06], 0.04
Gln/Gln	1	5	2.24 [0.22-23.11], 0.49	4.53 [0.34-59.67], 0.25
Arg/Gln+Gln/Gln	10	40	2.80 [0.89- 8.82], 0.08	2.83[0.89- 9.04], 0.08
XRCC1 codon 194				
Arg/Arg	5	56	1	1
Arg/Trp	9	38	2.65 [0.82- 8.53], 0.10	4.13 [1.13-15.12], 0.03
Trp/Trp	1	2	5.60 [0.43-73.08], 0.19	7.23 [0.50-103.89], 0.14
Arg/Trp+Trp/Trp	10	40	2.80 [0.89- 8.82], 0.08	3.06 [0.95- 9.83], 0.06
XRCC3 codon 241				
Thr/Thr	13	84	1	1
Thr/Met	2	12	1.07 [0.21- 5.37], 0.93	1.55 [0.27- 8.74], 0.62
HPV positive				
XRCC1 codon 399				
Arg/Arg	61	13	1	1
Arg/Gln	32	9	0.76 [0.29- 1.96], 0.57	0.71 [0.26- 1.93], 0.51
Arg/Gln+Gln/Gln	35	9	0.83 [0.32- 2.13], 0.70	0.82 [0.32- 2.14], 0.70
XRCC1 codon 194				
Arg/Arg	48	9	1	1
Arg/Trp	40	13	0.57 [0.22- 1.48], 0.25	0.57 [0.21- 1.55], 0.27
Arg/Trp+Trp/Trp	48	13	0.69 [0.27- 1.77], 0.44	0.69 [0.27- 1.78], 0.45

OR [95% CI] = Odds ratios [95%confidence interval] * adjusted with multiple logistic regression for age and smoking

Table 6 *XRCC* genotypes smoking and risk for SCCA

Genotype	Number of subjects		OR [95% CI], *p*-value	Adjusted OR* [95% CI], *p*-value
	Cases	Controls		
Non-smokers				
XRCC1 codon 399				
Arg/Arg	12	27	1	1
Arg/Gln	8	14	1.28 [0.43- 3.88], 0.65	4.61 [0.47-45.04], 0.19
Gln/Gln	2	3	1.50 [0.22-10.17], 0.68	14.87 [0.69-317.9], 0.08
Arg/Gln+Gln/Gln	10	17	5.78 [0.64-52.32], 0.12	1.32 [0.47-3.72], 0.59
XRCC1 codon 194				
Arg/Arg	13	21	1	1
Arg/Trp	5	23	0.35 [0.11-1.15], 0.08	0.12 [0.01- 1.0], 0.06
Arg/Trp+Trp/Trp	9	23	0.33 [6.06-1.94], 0.22	0.63 [0.22- 1.78], 0.63
XRCC3 codon 241				
Thr/Thr	20	39	1	1
Thr/Met	2	5	0.7 8[0.14- 4.38], 0.78	3.84 [0.33-44.41], 0.28
Smokers				
XRCC1 codon 399				
Arg/Arg	54	42	1	1
Arg/Gln	33	30	0.85 [0.45- 1.61], 0.63	1.06 [0.46- 2.41], 0.89
Gln/Gln	2	2	0.77 [0.11-575], 0.81	1.07 [0.08-14.29], 0.96
Arg/Gln+Gln/Gln	35	32	1.06 [0.47- 2.38], 0.88	0.85 [0.45- 1.59], 0.61
XRCC1 codon 194				
Arg/Arg	40	44	1	1
Arg/Trp	44	28	1.73 [0.91- 3.27], 0.09	1.68 [0.40- 3.83], 0.21
Trp/Trp	5	2	2.75 [0.50-14.97], 0.24	2.39 [0.28-20.08], 0.42

Arg/Trp+Trp/Trp	49	30	1.74 [0.78- 3.87], 0.17	1.79 [0.96- 3.35], 0.06
XRCC3 codon 241				
Thr/Thr	81	67	1	1
Thr/Met	8	7	0.9 [0.3- 2.7], 0.92	1.5 [0.4- 6.3], 0.52

OR [95% CI] = Odds ratios [95%confidence interval]
* adjusted with multiple logistic regression for age and HPV status

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Chapter 9

CERVICAL CANCER IN NORTHEASTERN THAILAND

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ABSTRACT

Cervical cancer remains the most common cancer among Thai women even though its incidence has decreased according to the age-standardized rate. Contrariwise, other data suggest the incidence rate of this cancer gradually increased in Northeast Thailand between 1992 and 2000. HPV infection is the major cause of cervical cancer and has been confirmed as a critical risk factor for the development of cancer in this region. The prevalence of HPV-16 infection was prominent, followed by HPV-18. The number of sexual partners and passive smoking history were identified as risks for HPV infection. As for passive smoking, tobacco specific carcinogens/mutagens may exist in the cervical mucus through smoking and/or in the semen of tobacco smoking partners. Cervical exposure to such carcinogens can reduce cervical immunity resulting in the persistence of HPV infection and also generating DNA lesions. It is hypothesized that certain genetic backgrounds, such as chemical metabolizing enzyme genes, may play roles in the development of this cancer.

Keywords: Cervical cancer, Northeastern Thailand, Risk factors, Cancer susceptibility.

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INTRODUCTION

Cancer is an important national health problem in Thailand. The incidence rates vary by region (*i.e.,* in the North, Northeast, South and Central (see Figure 1)) with the age-standardized incidence rates (ASR) being 149.2 in males and 125.0 in females between 1995 and 1997 (Martin and Patel, 2003). However, in Khon Kaen, Northeast Thailand, over the same period the ASR were 182.5 in males and 125.3 in females (Martin and Patel, 2003).

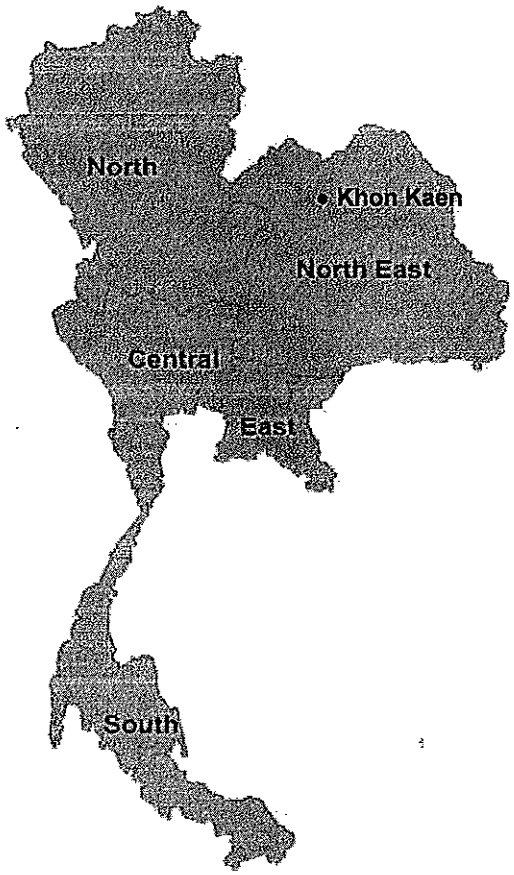

Figure 1. Map of Thailand.

Among cancers, cervical cancer is one of the most common in Thai women even though the age-standardized incidence rates indicate a decrease with 19.5 in 1995-1997 (Pengsaa and Jindawijak, 2003). The incidence rate of this cancer gradually increased in Northeastern Thailand between 1992 and 2000 (Pengsaa and Jindawijak, 2003), and remains the highest cause of mortality among females in the region (Vatanasapt *et al.,* 1998). Cervical cancer comprised one-fifth of new female cancer cases in the last two years. Two-thirds of patients with cervical cancer receive radiotherapy, while one-seventh underwent surgery at our regional University Hospital in Khon Kaen (Cancer Unit, 2004; 2005).

Cervical cancer can be detected at an asymptomatic stage using the Pap smear test; however, the test is not well accepted because of limited public health awareness. Thus, women attend hospital only after cervical cancer symptoms manifest, when they are difficult to treat so the prognosis is poor. This situation prompted us to carry out a survey to search for the risk factors for cervical cancer in Northeast Thailand to develop tools for early diagnosis and prevention of cervical cancer.

Human Papillomavirus Infection

Most cancers are multi-factorially developed through genetic background, environmental factors and lifestyle; all of which exert independently or in combination to cause cancer (Bishop, 1995; Hunter, 1991; Weinberg, 1991; 1989). Large series of molecular and epidemiological studies conducted over the last twenty years have indicated that cervical infection by certain human papillomavirus (HPV) types is a precursor event in the genesis of cervical cancer (zur Hausen, 1991; Schiffman and Castle, 2003).

The human papillomavirus is a virus sized 50-55 nm in diameter with small double-stranded DNA as its genome, which encodes a small number of genes (Doorbar and Strerling, 2001). The basic structure consists of a regulatory region, an early gene (E) region and a late gene (L) region. HPV types are identified by variation of E6, E7 and L1 parts of the HPV genome. A similarity of at least 90% in the nucleotide sequence in these three genes permits HPV to be classified as identical types.

To date, there are 100 known different types of HPV: 1) a low-risk group (such as HPV-6, -11, -26, -42 and -44); and, 2) a high-risk group (such as HPV-16, -18, -31, -33, -35, -39, 45, -51, -52, -56 and -58) (Minaguchi *et al.*, 1998). Cervical infection with certain subtypes of HPV, particularly of the high-risk group, closely correlates with the development of cervical cancer (Scheffner *et al.*, 1990; Schiffman and Castle, 2003; zur Hausen, 1991).

Strong evidence suggests that E6 and E7 HPV proteins contribute to the development of cervical cancer. They act together to stimulate keratinocytes and facilitate HPV DNA replication (Munger *et al.*, 2001). Once integration of the HPV genome into the host cells occurs, there is uncontrolled gene expression, which drives the cell cycle. In HPV-infected cells, the E7 protein binds with pRB turning E2F into an active form resulting in transcription of DNA replicated genes. Another HPV protein, E6 binds with p53 protein, which leads to degradation of the p53 protein (Scheffner *et al.*, 1990).

Epidemiological evidence indicates that infection with HPV is the major cause of cervical cancer (Monoz *et al.*, 1992; zur Hausen, 1991), such that worldwide HPV prevalence in cervical cancer is 99.7% (Walboomers *et al.*, 1999). Additionally, HPV infection was confirmed as a critical risk factor for the development of cervical cancer in Northeast Thailand. The prevalence of the high-risk group of HPV infection sub-strains -16, -18, -31, -33, -35, -52b and -58) was identified among 86.7% of squamous cell cervical cancers (SCCA) and increased the risk for development of SCCA by as much as 43.5-fold (Settheetham-Ishida *et al.*, 2005).

As expected, genotyping of HPV among SCCA patients in Northeast Thailand confirmed the high prevalence of HPV-16 infection (70.5%) followed by HPV-18 infection (23.1%), but

a relatively low prevalence of HPV-33 was noted (Settheetham-Ishida *et al.,* 2005). Moreover, HPV-16 is a critical risk factor for cervical squamous intraepithelial lesions (SIL) progression in this region (Settheetham-Ishida *et al.,* 2006). Compared to other populations vis-à-vis invasive SCCA, HPV-16 is the most prevalent genotype (Iwasawa *et al.,* 1996; Thomas *et al.,* 2001a), whereas preferential distribution of HPV genotype has been suggested causative for certain types of cervical cancer. HPV-18 is more closely associated with cervical adenocarcinoma and a poor prognosis (Kjaer and Brinton, 1993). Since female subjects of the study in Northeast Thailand were patients with SSCA, the high prevalence of HPV-16 is conceivable; however, the relatively high prevalence of HPV-18 in SCCA patients compared to the low prevalence in the controls should be emphasized. The difference in HPV genotype distribution between the controls and the patients was not statistically significant, indicating any high risk subtype may have potential for SCCA development. The accumulation of HPV-18 suggests a role for HPV-18 in the development of SCCA in Northeast Thailand.

The prevalence of a high-risk group for HPV infection varies in each region of Thailand; for example, in central and southern Thailand where the infection rate ranged between 3 and 14% among controls and between 74 and 89% among patients with cervical abnormalities (Lertworapreecha *et al.,* 1998; Sukvirach *et al.,* 2003; Thomas *et al.,* 2001a). In Northeast Thailand, prevalence was 13% for controls and 87% for patients (Settheetham-Ishida *et al.,* 2005), which is comparable to the other Thai populations (Lertworapreecha *et al.,* 1998; Sukvirach *et al.,* 2003; Thomas *et al.,* 2001) and strongly suggests the involvement of other risk factor(s) in the high incidence of cervical cancer in Northeast Thailand.

Genetic Polymorphism of *p53*

A substantial series of epidemiological evidence has indicated that infection with certain types of HPV is the major cause of cervical cancer (Monoz *et al.,* 1992; zur Hausen, 1991). However, only a small fraction of women infected with these virus strains develop the cancer indicating that HPV infection is necessary but not a sufficient condition for the cancer to develop, and that the presence of other risk factors – such as genetic backgrounds and lifestyle – is suspected.

The human p53 protein comprises 393 amino acids, which can be divided into several structural and functional domains, many of which contribute to the ability of p53 to function as a transcription factor (Levine, 1997; Haffner and Oren, 1995). The p53 acts as a tumor suppressor protein and cellular growth regulator: whereas p53 is not essential for basal cellular growth and development (Donehower *et al.,* 1992), it does play a critical role in the response of cells to stress; mediating a G1/S cell-cycle arrest and/or apoptosis following damage to the DNA. When the DNA is damaged, p53 levels rise and induce G1 cell-cycle arrest (Kastan *et al.,* 1991; Lowe *et al.,* 1993).

p53 protein is one of the most important proteins in the defense system against tumor development (Levine, 1997). Participation of the *p53* alterations including somatic mutations and germ-line polymorphisms in tumor development has been well documented (Andersson *et al.,* 2001; Zehbe *et al.,* 1999). A base substitution at codon 72 of the *p53* resulting in either

arginine (Arg) or proline (Pro) was identified as polymorphic in human populations (Beckman *et al.,* 1994). Codon 72 polymorphism (*Arg/Pro* allele) of the *p53* is widely distributed in various human populations (Helland *et al.,* 1998). One of the striking features of this polymorphism is efficient degradation of the Arg type of p53 by HPV E6 oncoprotein, and that carriers of the Arg type of p53 were about seven times more susceptible to the cervical cancer (Storey *et al.,* 1998).

After HPV infection, viral E6 protein binds to the cellular protein (Scheffner *et al.,* 1990) and causes dysfunction of the p53 protein activity. As recently reported, biological and biochemical differences – between the p53 protein with Arg or Pro at amino acid position 72 – indicate the possible presence of a different interaction pattern between either the p53 protein variant and the E6 protein of HPV property (Storey *et al.,*1998; Thomas *et al.,* 1999a, 1999b).

Thus, the *p53* codon 72 polymorphism has been nominated as a genetic risk factor for cervical cancer. However, the association was not significant and the susceptibility of the *Arg/Arg* genotype to cervical cancer was limited in Northeastern Thai women. This result is thus comparable to the studies of Korean, Japanese and Peruvian population which have Mongoloid background (Minaguchi *et al.,* 1998; Baek *et al.,* 2000; Klug *et al.,* 2001). The controversial results may be due to differences in ethnic background and in allele frequency. The allele frequency in Northeastern Thailand, shown in the present study, is similar to those reported in Central and Southern Thailand (Tiwawech *et al.,* 2003; Kietthubthew *et al.,* 2003).

The development of cervical cancer correlates with the presence of the high risk HPV E6 protein of the virus. It binds to and inactivates the tumor suppressor protein p53 and directs its degradation (Scheffner *et al.,* 1990; Werness *et al.,* 1990). Although inactivation of p53 by HPV is probably important for tumor development, no genetic factors have been conclusively identified that might predispose an infected individual to develop cervical carcinoma.

Thus, several studies evaluated the association between p53 and HPV in the development of cervical cancer. P53 protein is mutated in many human tumors; however, in cervical cancer, this mutation is rarely detected (Crook *et al.,* 1991; Scheffner *et al.,* 1991). Many mutated p53 proteins are not susceptible to E6-mediated degradation, while the wild type of p53 is susceptible (Marston *et al.,* 1994; Medcalf and Milner, 1993; Thomas *et al.,* 1995).

p53 is a tumor suppressor protein that modifies its cancer suppressing property (Storey *et al.,*1998; Thomas *et al.,* 1999a, 1999b), and the *p53* codon 72 polymorphism is observed with various allele frequencies worldwide. Storey *et al.* (1998) also suggested that women homozygous for the *Arg* allele were about seven times more susceptible to cervical cancer than *Arg/Pro* heterozygous women and concluded that the polymorphism is involved in the development of cervical cancer, especially in the presence of HPV infection.

Storey *et al.* (1998) showed that the *p53* polymorphism at codon 72 *p53* affects protein susceptibility to HPV-16 and 18 E6-induced degradation *in vivo*; Arg type is more susceptible than Pro type. In cell lines transfected with E6 expressing plasmids, E6-mediated degradation of the p53 with Arg was more effective than the one carrying Pro.

The high risk type of p53 with Arg for cervical cancer was supported by several epidemiological studies for European (Makni *et al.,* 2000; Yang *et al.,* 2001; Zehbe *et al.,* 1999); however, this finding was contradicted by British (Rosenthal *et al.,* 1998), Japanese

(Minaguchi *et al.*, 1998), Korean (Kim *et al.*, 2001) and Black South African (Pegoraro *et al.*, 2000) studies. In addition, the distribution of the two p53 variants in healthy women and women with HPV positive cervical cancer suggests that women with the *Arg/Arg* are at a higher risk of HPV-associated cervical cancer than *Pro/Pro* or *Arg/Pro* (Storey *et al.*, 1998), although several studies found no statistically significant differences in the distribution of *p53* codon 72 genotypes between normal women and patients with cervical cancer (Minaguchi *et al.*, 1998; Yamashita *et al.*, 1999; Beak *et al.*, 2000; Kim *et al.*, 2001; Gustafsson *et al.*, 2001). Since the distribution of the *p53* codon 72 genotype varies according to ethnicity (Helland *et al.*, 1998), it is thus difficult to generalize from conclusions obtained for a given population.

In our study in Northeast Thailand, no significant association was found between the genotype distribution of the *p53* codon 72 polymorphism and the HPV infection; even though it seems that the odds ratio (OR) were increased in individuals homozygous for the *Arg* allele compared with those homozygous for the *Pro* allele, in particular after being adjusted for other risk factors. There was no significant association between genotype distribution of the *p53* codon 72 polymorphism and the HPV infection. Since polymorphism of the *p53* itself – as well as in combination with HPV infection – may not be a genetic risk for cervical cancer of women in Northeastern Thailand, more attention should be paid to other risk factors such as sexual behaviors and smoking.

Lifestyle

Although human papillomavirus (HPV) infection is a major risk – in particular for squamous cell cervical cancer – most infected women do not develop invasive cervical cancer. This means HPV infection is not a sufficient factor, and cofactors must therefore play roles in developing cancer. Transformation of lifestyle can also modify prevalence of leading cancers. This kind of public health approach has reached Northeast Thailand; however, the high incidence of cervical cancer in the region persists.

Epidemiologic evidence suggests that other factors such as sexual behaviors are risk factors for cervical cancer in Thailand (Punyaratabunduh *et al.*, 1982; Thomas *et al.*, 1996). In our study of Northeast Thai women, we confirmed that the nominated risks for cervical carcinoma in other studies (Tachezy *et al.*, 1999; Sriamporn *et al.*, 1997; Monoz, 2002; Skegg, 2002) – number of sexual partners, age at the first sexual intercourse and number of parities (Settheetham-Ishida *et al.*, 2004a) – also increased the risk. These parameters related to sexual behaviors increased the risk of cervical cancer between 2.18 and 2.76-fold. If the first sexual intercourse occurs in a young girl – in whom the cervix is vulnerable because of inadequate production of cervical mucus that acts as a protective barrier against infectious agents – HPV infection occurs easily and leads to a higher risk of cervical cancer (Apter *et al.*, 1993).

Sexually transmitted diseases (such as syphilis, herpes simplex type 2 and chlamydia infection) are associated with the development of invasive cervical cancer (Williams *et al.*, 1994; Kahn *et al.*, 2002), as are contraceptives, particularly the pill (Skegg, 2002; Moreno,

2002); however, none of these factors were confirmed in women living in Northeast Thailand (Settheetham-Ishida *et al.*, 2004a).

Smoking has also been thought to be a risk factor for cervical cancer (Prokopczyk *et al.*, 1997); however, the OR was not increased among smoking groups in Northeastern Thai women (Settheetham-Ishida *et al.*, 2004a). A weak association of smoking and risk of invasive cervical cancer was reported from Central Thailand (Thomas *et al.*, 2001). Thomas *et al.* (2001) suggested that even if smoking is a cofactor for cervical cancer development, it operates prior to the development of *in situ* disease and is not an important determinant of risk in Thailand. This is especially so because smoking is culturally unacceptable for Thai women. Increased risks were observed when the partner smoked or had smoking history, confirming that secondary smoking is a risk factor for cervical cancer, especially among high-risk HPV positive women (Coker *et al.*, 2002). Smoking directly promotes the development of lung cancer in males, while it does the same indirectly for cervical cancer in females (National Cancer Institute, 2001).

As mentioned above, cervical infection with certain HPV types is a precursor event in the genesis of cervical neoplasia (zur Hausen, 1991; Schiffman and Castle, 2003). Oncogenic high-risk group of HPV infection was identified among 86.7% of patients with SCCA and increased the risk for cervical cancer development as high as 43.5-fold in Northeastern Thai women (Settheetham-Ishida *et al.*, 2005). Multiple sexual partners and partner's smoking habit were nominated as the risks for HPV infection. Having multiple sexual partners increased the risk for high-risk type HPV infection 3.94-fold (Settheetham-Ishida *et al.*, 2004b). This is comparable to the data obtained in Central, Northern and Southern Thailand (Thomas *et al.*, 1996; Sukvirach *et al.*, 2003). Women who have multiple sexual partners easily contract HPV from their partners (Thomas *et al.*, 2001a). Moreover, extramarital sexual contact of the husband causes HPV infection in women who are monogamous (Thomas *et al.*, 2001b).

Any smoking experience of the partner was confirmed as a risk for cervical cancer in Thai women living in the Northeast (Settheetham-Ishida *et al.*, 2004a). As for the HPV infection, an increased risk was observed when the subject had (a) current smoking partner(s) (Settheetham-Ishida *et al.*, 2004b). This confirms that passive or secondary smoking (Coker *et al.*, 2002) as a risk factor for high-risk type HPV infection. The association between sexual partner's smoking and HPV infection is explained by either inhalation of environmental smoke or exposure to smoke-related mutagens/carcinogens in semen (Tokudome, 1997) and also smoke-related agents have been detected in cervical mucus (Prokopczyk *et al.*, 1997). Smoking, thus, might cause a local immunological depletion and smoke components could favor HPV persistence (Poppe *et al.*, 1995; Lazcano-Ponce *et al.*, 2001). Moreover, smoking results in persistence of cervical HPV infection and lowers potential for clearing oncogenic infection (Giulian *et al.*, 2002). Since women in Northeast Thailand rarely smoke, most of the exposure to smoke is passive but the cancer promotion is still active.

Some other factors such as sexual behaviors (particularly, having more than one sexual partner and younger age at the first sexual intercourse) and smoking also increased risk for malignant types of HPV infection and cervical squamous intraepithelial lesions (SIL) development. These risk factors may enhance the effects of HPV infection on cervical hyperplasia and transformation (Settheetham-Ishida *et al.*, 2006).

Increased risk for age at the first sexual intercourse, number of parities, long-term use of oral contraceptives and sexual partner's smoking habit was observed even though they did not statistically increase the risk for HPV infection. Since these factors are associated with risk for cervical cancer (Settheetham-Ishida *et al.*, 2004a, 2004b), together with multiple sexual partners and partner's smoking habit, they may all contribute to the development of cervical cancer. To decrease the incidence of cervical cancer, prevention of HPV infection is essential as is limiting the number of sexual partners, especially those with a smoking habit.

Polymorphisms in Detoxification Enzymes

It has been suggested that tobacco smoking is associated with the risk of HPV infection and cervical cancer, including in Northeastern Thai women (Settheetham *et al.*, 2004a, 2004b). The presence of carcinogens – such as nicotine, cotinine and tobacco-specific nitrosamines – have been detected in the cervical mucus of smokers. Inhaled tobacco-derived components are transported to cervical site(s) where they can damage cellular DNA (Prokopczyk *et al.*, 1997; McCann *et al.*, 1992). The tobacco smoke constituents are modified by metabolizing enzymes and may promote malignant cellular growth (Prokopczyk *et al.*, 1997). Even though smoking is a risk for cervical cancer, not all smokers develop cervical cancer. Molecular studies have identified polymorphic gene products that are associated with the metabolism of tobacco smoke pro-carcinogens and possibly with susceptibility to cancer.

Glutathione S-transferase (GST) is related to human phase II detoxification enzymes, and at least five classes (alpha, mu, pi, sigma and theta class) of cytosolic GST have been identified. However, only enzymes in three classes – including GSTM (mu), GSTP (pi) and GSTT (theta) – play key roles in the detoxification of carcinogenic electrophiles of aflatoxin and polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke (*i.e.*, benzo[*a*]pyrene and other procarcinogens of PAHs). The mode of action is through the activation and detoxification of tobacco carcinogens, thus one might expect the relation of polymorphism and risk to be stronger among smokers.

GSTM1 facilitates the excretion of a wide range of carcinogens, reactive oxygen species and chemotherapeutic agents with a variety of substrate specificities (Rebbeck, 1997). The absent of the homozygous allele of the *GSTM1* gene (*GSTM*-null genotype) results in a complete loss of enzyme activity to bind with genotoxic substrates including epoxides derived from aflatoxin and PAHs (Hayes and Pulford, 1995). It is believed that individuals with *GSTM1*-null genotype are more prone to develop nasopharyngeal carcinoma than those with the *GSTM1*-present genotype because of the lack of function to detoxify the ultimate form of carcinogens (Tiwawech *et al.*, 2005).

Accumulative data from molecular epidemiological studies demonstrated that individuals with *GSTM1*-null genotype are susceptible to cancer in various organs including lung, bladder, skin, oral, liver, gastric, colorectal, prostrate, breast, ovary, cervix and nasopharynx (Nazar-Stewart *et al.*, 1999; Sweeney *et al.*, 2003; Lee *et al.*, 2002; Heagerty *et al.*, 1994; Kietthubthew *et al.*, 2001; Deng *et al.*, 2001; Setiawan *et al.*, 2000; Gawronska-Szklarz *et al.*, 1999; Autrup *et al.*, 1999; van der Hel *et al.*, 2003; Spurdle *et al.*, 2001; and Sierra-Torres *et al.*, 2003). However, results of many other studies dealing with the association between

GSTM1 polymorphism in some of these cancers have been contradictory (Kelsey, *et al.*, 1997; Kim *et al.*, 2002; Zheng *et al.*, 2003; Lallas *et al.*, 2000; and Cheng *et al.*, 2003).

We then screened for the *GSTM1* polymorphism in our case-control subjects. *GSTM1*-null genotype did not significantly increase the susceptibility to cervical cancer development but just showed a tendency; this was also confirmed among women exposed to passive smoking (Table 1). It is thus presumed that specificity and inter-individual variation in the expression level of metabolizing enzymes in cervical tissue may be involved in certain genetic backgrounds, of the chemical metabolizing enzyme genes, related to the development of cervical cancer. Other phase II detoxifying enzymes, such as GSTP and GSTT as well as phase I detoxifying enzymes namely P450 (CYP) family that are responsible for the metabolic activation of carcinogens will be our next target genes. Some of these genes are known to be polymorphic in Thai population and a polymorphic locus or combinations of polymorphic loci may have potentials to play critical roles in inter-individual genetic diversity relating to cervical cancer susceptibility.

Table 1. Frequency of GSTM1 Genotypes

	GSTM1 Genotypes, (n)		OR [95% CI]
	Normal	null	
Subjects			
Controls	32	54	
Cases	34	44	1.3 [0.67-2.56]
Passive Smokers			
Controls	19	30	
Cases	28	34	1.3 [0.57-2.99]

CONCLUSION

HPV infection is identified as a critical risk factor for the development of cervical cancer in Northeast Thailand. Among high-risk HPV types, HPV-16 as well as other malignant types have more or less equal potential for the development of SCCA. Multiple HPV infection was observed in a total of 18 out of 91 individuals; however, only one-third of them had declared to have multiple sex partners. Evidently, a partners' sexual behavior is critical in the dissemination of HPV in women. The polymorphism of the *p53* itself – as well as in combination with HPV infection – may not be a genetic risk for cervical cancer.

Regarding the other risk factors such as sexual behaviors and smoking, more attention should be paid. Since sexual behaviors, sexually transmitted diseases, the use of contraceptives and smoking may serve as cofactors to increase the risk for cervical carcinoma in the presence of HPV. In order to achieve eradication of HPV infection and the prevention of cervical cancer in this region, more attention should be paid to the presence of other risk factors such as sexual behavior and smoking habit.

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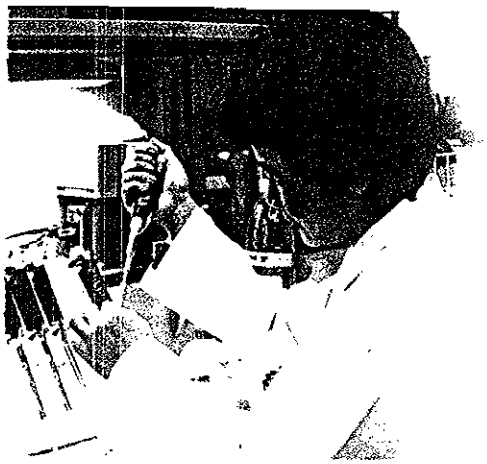
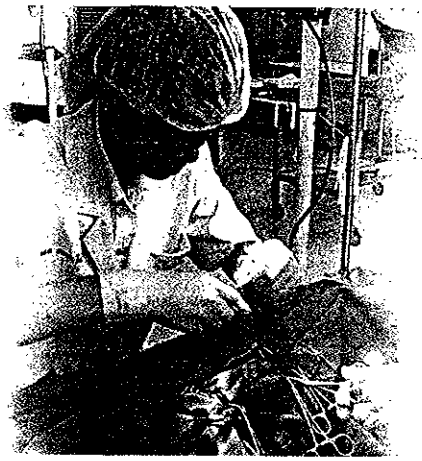
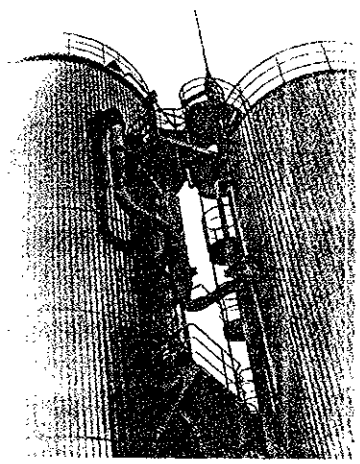






- เครื่องต้นแบบผลิตแท่งปุ๋ยอินทรีย์เพื่อสวนผลไม้
- ผศ.ดร.สันติ แม้นศิริ นักวิทยาศาสตร์รุ่นใหม่ สาขาฟิสิกส์ ประจำปี 2550
- นวัตกรรมถังปฏิกรณ์กู้พลังงานกลับคืนจากน้ำเสียด้วยประสิทธิภาพสูง
- อุบัติการณ์โรคพยาธิใบไม้ตับและมะเร็งท่อน้ำดี
- ภัยใกล้ตัวจาก “มะเร็งปากมดลูก”

วรรณภา อิชิดะ ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

ภัยใกล้ตัวจาก "มะเร็งปากมดลูก"

มะเร็งปากมดลูกเป็นมะเร็งที่สำคัญอันดับหนึ่งของสตรีไทย รวมทั้งประชากรในภาคตะวันออกเฉียงเหนือ จากข้อมูลของกรมการแพทย์พบว่ามะเร็งปากมดลูกเป็นโรคที่ทำให้สตรีไทยเสียชีวิตมากเป็นอันดับ 1 ประมาณปีละ 3 พันราย และพบผู้เป็นมะเร็งปากมดลูกเพิ่มขึ้นปีละประมาณ 6 พันราย ส่วนใหญ่มีอายุเฉลี่ยประมาณ 45 ปี จากรายงาน 10 ปีย้อนหลัง (พ.ศ. 2538-2549) ของหน่วยมะเร็งคณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น พบผู้ป่วยที่เป็นมะเร็งปากมดลูกสูงมากเป็นอันดับหนึ่งของโรคมะเร็งทั้งหมดในสตรีที่มารับการรักษา ณ โรงพยาบาลศรีนครินทร์ มหาวิทยาลัยขอนแก่น ในปีที่ผ่านมา (พ.ศ.2549) พบผู้ป่วยที่เป็นมะเร็งปากมดลูกที่มารับการรักษา ณ โรงพยาบาลศรีนครินทร์ มหาวิทยาลัยขอนแก่นมากถึง 317 ราย หรือคิดเป็นร้อยละ 14.4 จากสตรีที่มารับการรักษา ด้วยโรคมะเร็งทั้งหมดจำนวน 2,199 ราย ผู้ป่วยที่เป็นมะเร็งปากมดลูกส่วนใหญ่ร้อยละ 92.5 มีอายุอยู่ในช่วง 35-70 ปี โดยช่วงอายุที่พบมากที่สุดอยู่ระหว่าง 45-55 ปี (ร้อยละ32.2) ธรรมชาติของโรคนี้เริ่มจากการมีเซลล์เปลี่ยนแปลง จนกลายเป็นเซลล์มะเร็งปากมดลูกใช้เวลาเกือบ 10 ปี มะเร็งปากมดลูกเป็นโรคที่สามารถรักษาให้หายขาดได้ ถ้าพบเซลล์ปากมดลูกผิดปกติในระยะเริ่มแรกจากการตรวจคัดกรองมะเร็งปากมดลูก เช่น วิธีแป๊บสเมียร์ (Pap smear) อย่างไรก็ตาม การตรวจแป๊บสเมียร์ยังไม่สามารถครอบคลุมทุกพื้นที่ในประเทศไทยได้ ดังนั้นผู้ป่วยส่วนใหญ่ที่มาพบแพทย์จึงเป็นผู้ที่เป็นมะเร็งปากมดลูกระยะลุกลามและมีอาการมากแล้ว ทำให้การรักษายุ่งยากและสิ้นเปลืองค่าใช้จ่ายเพิ่มขึ้น การรักษาอาจจะหายหรือไม่ขึ้นอยู่กับความรุนแรงของโรค ในรายที่โรคลุกลามมากอาจทุกข์ทรมานและถึงแก่ชีวิต ในปัจจุบันเป็นที่แน่ชัดว่าการติดเชื้อไวรัสฮิวแมนแป๊ปโลมา (human papilloma virus หรือ HPV) หรือเชื้อไวรัสหงอนไก่ ซึ่งสามารถติดต่อกันทางเพศสัมพันธ์ เป็นสาเหตุหลักที่ทำให้เกิดมะเร็งปากมดลูก อย่างไรก็ตามการติดเชื้อไวรัสหงอนไก่ ไม่ได้ทำให้เกิดมะเร็งปากมดลูกทุกราย ซึ่งแสดงให้เห็นว่าน่าจะมีปัจจัยเสี่ยงอื่นอีกที่เป็นสาเหตุทำให้เกิดความผิดปกติของเซลล์ปากมดลูก เช่น ลักษณะทางพันธุกรรมและพฤติกรรมเสี่ยง เป็นต้น ดังนั้นการทราบปัจจัยเสี่ยงที่เป็นสาเหตุของการเกิดมะเร็งปากมดลูกจึงช่วยในการวางแผนการป้องกันรวมถึงเป็นแนวทางในการวางแผนการรักษาเซลล์ปากมดลูกที่เริ่มผิดปกติจนกระทั่งเป็นมะเร็งปากมดลูกที่ยังไม่ลุกลามของประชากรในภาคตะวันออกเฉียงเหนือนี้ได้

คณะวิจัยโดยมีรองศาสตราจารย์ ดร.วรรณภา อิชิดะ เป็นหัวหน้าโครงการวิจัย จึงได้ทำการศึกษาเพื่อสืบค้นปัจจัยที่มีผลต่อการเกิดมะเร็งปากมดลูกของสตรีที่อยู่ในภาคตะวันออกเฉียงเหนือโดยมีความมุ่งหวังเพื่อ

1. ศึกษาความสัมพันธ์ของปัจจัยเสี่ยงด้านพฤติกรรมและลักษณะพันธุกรรม โดยเลือกศึกษา จีนพี 53 ที่ตำแหน่งโคดอน 72 ต่อการเกิดมะเร็งปากมดลูก ซึ่งจีนพี 53 มีหน้าที่ควบคุมการแบ่งเซลล์ ถ้าจีนพี53ทำงานบกพร่อง เซลล์จะแบ่งตัวเพิ่มขึ้นมากจนกลายเป็นมะเร็งได้ และที่ตำแหน่งโคดอน 72 ของจีนพี 53 ในคนปกติมีลักษณะที่แตกต่างกัน ซึ่งเชื่อว่ามีผลทำให้แต่ละคนสามารถเกิดเป็นมะเร็งบางชนิดได้แตกต่างกันด้วย

2. ศึกษาความสัมพันธ์ของปัจจัยเสี่ยงด้านพฤติกรรมและลักษณะพันธุกรรมของจีนพี53 ที่ตำแหน่งโคดอน 72 ต่อการติดเชื้อไวรัสหงอนไก่

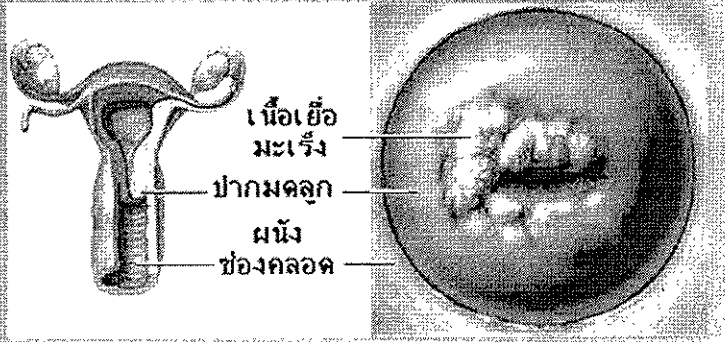
ผลการศึกษาพบว่า

1. การเกิดมะเร็งปากมดลูกไม่มีความสัมพันธ์กับลักษณะทางพันธุกรรมของจีนพี 53 ที่ตำแหน่งโคดอน 72 แต่มีความสัมพันธ์กับการมีเพศสัมพันธ์ครั้งแรกเมื่ออายุน้อยกว่า 17 ปี การมีคู่นอนมากกว่า 1 คน การคลอดบุตรหลายครั้ง รวมทั้งการสูบบุหรี่ของสามี

2. การติดเชื้อไวรัสหงอนไก่ ชนิดความเสี่ยงสูงเช่น ชนิด 16 18 31 33 52 และ 58 จะมีผลทำให้มีความเสี่ยงต่อการเกิดมะเร็งปากมดลูกถึง 44 เท่า การติดเชื้อไวรัสหงอนไก่ ไม่มีความสัมพันธ์กับลักษณะทางพันธุกรรมของจีน พี. 53 ที่ตำแหน่งโคดอน 72 แต่มีความสัมพันธ์กับการมีเพศสัมพันธ์ครั้งแรกเมื่ออายุน้อยกว่า 17 ปี รวมทั้งการสูบบุหรี่ของสามี

จากการศึกษาวิจัยนี้พบว่า ปัจจัยด้านพันธุกรรมของจีน พี 53 ที่ตำแหน่งโคดอน 72 อาจจะไม่ใช่สาเหตุที่ทำให้เกิดมะเร็งปากมดลูก แต่ปัจจัยเสี่ยงด้านพฤติกรรม น่าจะเป็นปัจจัยหลักที่มีผลต่อการติดเชื้อไวรัสหงอนไก่ และ/หรือการเกิดมะเร็งปากมดลูก ดังนั้นการหลีกเลี่ยงปัจจัยเสี่ยงดังกล่าว ได้แก่ การมีเพศสัมพันธ์ครั้งแรกเมื่ออายุน้อยกว่า 17 ปี การมีคู่นอนมากกว่า 1 คน การคลอดบุตรหลายครั้ง รวมถึงการสูบบุหรี่ของสามี น่าจะช่วยป้องกันและลดการติดเชื้อไวรัสหงอนไก่ รวมทั้งลดการเกิดมะเร็งปากมดลูกลงได้ การตรวจแป๊บสเมียร์อย่างสม่ำเสมอเพื่อหาเซลล์ปากมดลูกที่เริ่มผิดปกติจะเป็นอีกหนทางหนึ่งที่จะช่วยป้องกันการเกิดมะเร็งปากมดลูกและสามารถลดอัตราการตายจากมะเร็งปากมดลูกระยะลุกลามได้

มีข้อมูลที่สนับสนุนผลที่ได้การศึกษาถึงผลของบุหรี่ต่อการติดเชื้อไวรัสหงอนไก่ และมีผลต่อการเกิดมะเร็งปากมดลูก โดยพบสารก่อมะเร็งหลายชนิดที่มีในบุหรี่ เช่น นิโคติน (nicotine) โคตินิน (cotinine) ไนโตรซามีน (tobacco-specific nitrosamines) และสารประกอบไฮโดรคาร์บอนอื่นๆ ในช่องคลอดของสตรีและในน้ำกามของบุรุษที่มีการสัมผัสบุหรี่ ซึ่งสารก่อมะเร็งในน้ำกามของบุรุษที่สูบบุหรี่สามารถคัดหลั่งเข้าสู่ช่องคลอดสตรีได้ เมื่อมีเพศสัมพันธ์ สารพิษเหล่านี้มีฤทธิ์ลดภูมิต้านทานของร่างกายในการกำจัดเชื้อโรค รวมทั้ง เชื้อไวรัสหงอนไก่ ที่ช่องคลอด ปากมดลูก และมดลูกด้วย และยังมีผลต่อการเปลี่ยนแปลงของสารพันธุกรรม (ดีเอ็นเอ) ในเซลล์ด้วย ทำให้มีการสร้างเซลล์และแบ่งเซลล์ใหม่ในระบบสืบพันธุ์ผิดปกติอันนำไปสู่การเกิดมะเร็งในที่สุด อย่างไรก็ตามการสัมผัสบุหรี่ไม่ได้ทำให้สตรีทุกรายกลายเป็นมะเร็งปากมดลูก ดังนั้นน่าจะมีปัจจัยส่วนอื่นเข้ามาเกี่ยวข้องในกระบวนการเปลี่ยนแปลงนี้ โดยเฉพาะกลไกการทำลายสารก่อมะเร็งในร่างกายซึ่งเกี่ยวข้องกับเอนไซม์หลายชนิด คณะผู้วิจัยจึงได้ทำการศึกษาต่อยอดถึงความแตกต่างของจีนในการสร้างเอนไซม์ที่เกี่ยวข้องกับการทำลายสารพิษที่เป็นสารก่อมะเร็งในบุหรี่ ทั้งนี้ได้เลือกทำการการศึกษาจีนของเอนไซม์ Glutathione S-transferase (GST) ในสตรีที่ได้รับบุหรี่ ทั้งด้วยการได้รับบุหรี่โดยตรงจากการสูบเองหรือโดยทางอ้อมจากการสูดเอาควันบุหรี่จากสามีที่สูบบุหรี่ต่อการเกิดเร็งปากมดลูก โดยจีน GST มีหลายชนิด ได้แก่ alpha, mu, pi, sigma and theta ผู้วิจัยจึงเลือกศึกษา GST-mu (GSTM) เนื่องจากข้อมูลการศึกษาในต่างประเทศพบว่า GSTM มีสองรูปแบบคือ GSTM-null หรือ GSTM-present มีผลต่อการสร้างโปรตีนที่ทำหน้าที่เป็นเอนไซม์ GSTM ที่แตกต่างกัน ทำให้มีความสามารถในการทำลายสารก่อมะเร็งที่มีในบุหรี่แตกต่างกัน อันส่งผลต่อการเกิดมะเร็งที่แตกต่างกันในสตรีที่ได้รับบุหรี่ โดยทำการศึกษาเบื้องต้นในอาสาสมัครจำนวน 200 ตัวอย่างที่เป็นมะเร็งปากมดลูกชนิดลุกลามและสตรีที่มีสุขภาพดี ผลการศึกษาเบื้องต้นนี้ ยังไม่พบความแตกต่างของจีน Glutathione S-transferase (GST) ชนิด GSTM ทั้งสองรูปแบบ ในการเพิ่มความเสี่ยงต่อการติดเชื้อไวรัสหงอนไก่ และ/หรือการเกิดมะเร็งปากมดลูก แต่ในสตรีที่ได้รับบุหรี่นั้นสตรีที่มีจีนนี้ในรูป GSTM-null จะทำให้มีโอกาสเสี่ยงต่อการการติดเชื้อไวรัสหงอนไก่ และ/หรือการเกิดมะเร็งปากมดลูกได้มากกว่าการมีจีนในรูป GSTM- present แม้ว่าไวรัสหงอนไก่เป็นไวรัสที่ติดต่อทางเพศสัมพันธ์ในมนุษย์ ไวรัสหงอนไก่ หรือไวรัสเอชพีวีชนิด 16 และ 18 เป็นต้นเหตุมะเร็งปากมดลูกถึงร้อยละ 70 มะเร็งชนิดที่คร่าชีวิตผู้หญิงประมาณ 3 แสนคน ทั่วโลกต่อปี ซึ่งรวมถึงประมาณ 4 พันคนในสหรัฐ ขณะที่สถิติของไทยในปี 2539 มีผู้ป่วยมะเร็งปากมดลูก 6,268 ราย และปีที่แล้ว (2549) มีผู้เสียชีวิตถึง 1,632 ราย ขณะเดียวกันก็ได้รับข่าวดีจากบริษัทยาต่างประเทศที่ได้ประกาศผลสำเร็จในการทดสอบวัคซีนในมนุษย์กว่า 10,000 คน ซึ่งสามารถป้องกันการเกิดมะเร็งปากมดลูกได้ถึง 100 % และผลทดลองกับคนไทย ต้องรอสรุปปลายปีนี้ ซึ่งแพทยสภาได้ให้ข้อมูลเพิ่มเติมว่าวัคซีนมะเร็งปากมดลูกมีประสิทธิภาพในการป้องกันการติดเชื้อเอชพีวี 16 และ 18 ซึ่งเป็นไวรัสตัวการก่อโรคมะเร็งปากมดลูกที่สำคัญ และสามารถช่วยแก้ปัญหาโรคมะเร็งอันดับหนึ่งที่คร่าชีวิตผู้หญิงไทยได้ ขณะนี้ยังอยู่ระหว่างการประเมินความคุ้มทุนที่จะประกาศให้เป็นแผนรณรงค์ จัดภูมิคุ้มกันทั่วประเทศต่อไป ความสำเร็จในการทดลองวัคซีนมะเร็งปากมดลูก นับเป็นข่าวดีสำหรับสตรีนับ 500,000 รายทั่วโลกที่ป่วยเป็นมะเร็งชนิดนี้ โดยในแต่ละปีจะมีสตรีที่เสียชีวิตด้วยมะเร็งปาก มดลูกถึง 300,000 ราย แม้ว่าวัคซีนดังกล่าวสามารถ ป้องกันการติดเชื้อไวรัสเอชพีวีชนิด 16 และ 18



ได้ถึง 100 % ด้วยการฉีดวัคซีน 3 โดส โดยใช้ ระยะเวลาการติดตามผลนาน 24 เดือน แต่การเกิดมะเร็งปากมดลูกไม่ได้มีสาเหตุจากการติดเชื้อไวรัสเอชพีวีทั้งหมด สตรีที่ป่วยเป็นมะเร็งปากมดลูกอีกประมาณร้อยละ 30 เกิดจากสาเหตุอื่นนอกเหนือจากการติดเชื้อไวรัสเอชพีวีชนิด 16 และ 18 ดังนั้นปัจจัยเสี่ยงต่อการเกิดมะเร็งปากมดลูกจึงยังรอการค้นหา เพื่อนำมาซึ่งการป้องกันที่มีประสิทธิภาพเพื่อสุขภาพที่ดีของสตรีต่อไป

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วารสาร

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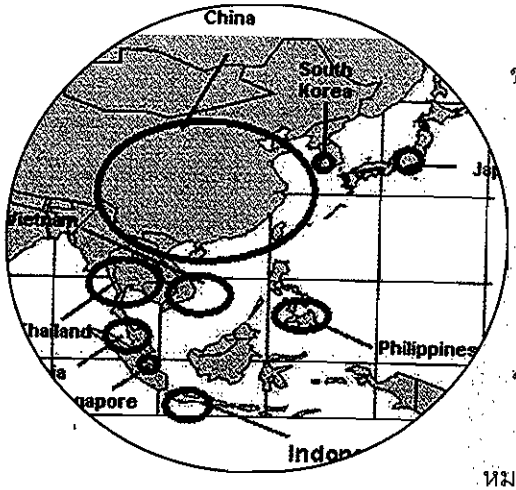
"มข. ปลูกถ่าย Stem cell สำเร็จแห่งแรกในภาคอีสาน"

- นวนาทัศนะ : มหาวิทยาลัยขอนแก่นจะเป็นมหาวิทยาลัยแห่งการวิจัยได้หรือไม่
- การปลูกเซลล์ต้นกำเนิดช่วยชีวิตผู้ป่วยมะเร็งสำเร็จแห่งแรกในภาคอีสาน
- ความพลากหลายของจีนกับการเกิดมะเร็งปากมดลูก
- การผลิตและพัฒนาผลิตภัณฑ์อาหารเช้าจากข้าวเม่า
- พลังแห่งความคิด...พลังแห่งการกำหนดสุขหรือทุกข์ของมนุษย์ (2)



ในต่างจังหวัด ยื่นขอที่ศาลากลางจังหวัดในจังหวัดนั้นๆ

2. ซื้อโปรแกรมคอมพิวเตอร์ที่ถูกต้องตามกฎหมายเท่านั้นมาติดตั้ง หากต้องการประหยัดเงินในส่วนนี้โปรดติดต่อศูนย์เทคโนโลยีอิเล็คโทรนิคส์และคอมพิวเตอร์แห่งชาติ (เนคเทค) โทร. 0-2642-5001-10 ซึ่งมีโปรแกรมลีนุกส์ โปรแกรมโอเพ่นออฟฟิศ และโปรแกรมปลาดาวออฟฟิศของบริษัทชันไมโครซิสเต็มส์ (ประเทศไทย) จำกัด ให้ใช้เว็บไซต์ www.Pladao.com

3. โปรแกรมต่างๆที่จะนำมาให้บริการควรซื้อลิขสิทธิ์ในการเผยแพร่จากเจ้าของลิขสิทธิ์เพื่อป้องกันการละเมิดและถูกจับกุม

4. ภายหลังปิดร้านทุกวันควรตรวจเช็คเครื่องคอมพิวเตอร์ของท่านว่ามีโปรแกรมคอมพิวเตอร์มาแปลกปลอมมาติดตั้งไว้ในเครื่องคอมพิวเตอร์ของท่านหรือไม่ หากมีให้รีบลบออกเพื่อป้องกันการถูกจับกุม

5. ควรติดตั้งป้ายภายในร้านว่าที่ร้านของท่านใช้โปรแกรมคอมพิวเตอร์มีอะไรบ้าง และห้ามลูกค้านำโปรแกรมแปลกปลอมมาติดตั้ง

6. ติดตั้งวีดีโอวงจรปิดภายในร้านเพื่อบันทึกหน้าตาลูกค้าและใช้เป็นพยานหลักฐาน ในกรณีที่ลูกค้านำโปรแกรมอื่นมาโหลดใส่โดยเป็นเหตุให้ถูกจับกุม

7. ควรบันทึกชื่อและที่อยู่ลูกค้าและหมายเลขเครื่องคอมพิวเตอร์ที่เล่น เพื่อใช้เป็นพยานหลักฐาน หากมีความจำเป็น

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1. www.ipthailand.org.th

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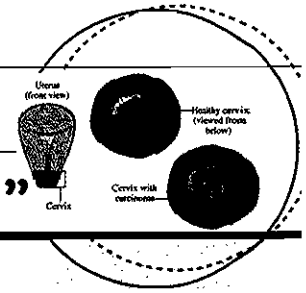
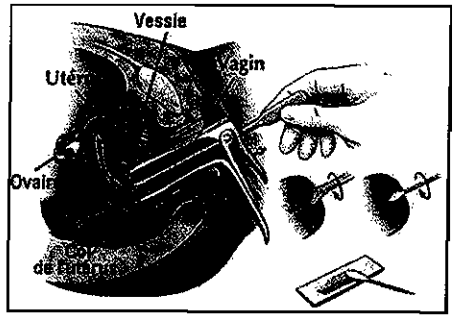
บทความวิจัย

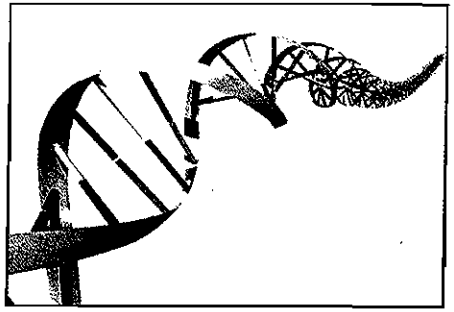
รศ.ดร.วรรณภา ชีวีตะ คณะแพทยศาสตร์
มหาวิทยาลัยขอนแก่น

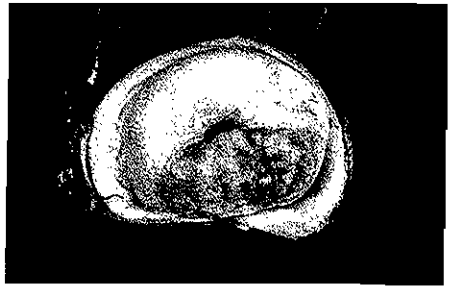
ความหลากหลายของจีน กับการเกิด "มะเร็งปากมดลูก"



"มะเร็งปากมดลูก" เป็นโรคมะเร็งสำคัญชนิดหนึ่งของสตรีไทย จากข้อมูลของกรมการแพทย์พบว่ามะเร็งปากมดลูกเป็นโรคที่ทำให้สตรีไทยเสียชีวิตมากเป็นอันดับ 1 ประมาณ ปีละ 3,000 ราย และพบผู้เป็นมะเร็งปากมดลูกเพิ่มขึ้นปีละประมาณ 6,000 ราย อายุเฉลี่ยของผู้ป่วยประมาณ 45 ปี จากรายงานของหน่วยมะเร็ง คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น พบว่ามีผู้ป่วยของโรคมะเร็งในสตรีที่มารับการรักษา ณ โรงพยาบาลศรีนครินทร์ มหาวิทยาลัยขอนแก่น โดย ในปี พ.ศ. 2550 มีผู้ป่วยมะเร็งปากมดลูก ที่เข้ามารักษามากถึง 321 ราย หรือคิดเป็นร้อยละ 14.3% ของจำนวนสตรีที่เป็นโรคมะเร็งทั้งหมด จำนวน 2,245 ราย การเกิดมะเร็งปากมดลูกเริ่มจากเซลล์ค่อย ๆ มีการเปลี่ยนแปลงจากเดิมจนกลายเป็นเซลล์ในที่สุด ซึ่งใช้เวลาประมาณ 10 ปี มะเร็งปากมดลูกเป็นโรคที่สามารถรักษาให้หายขาดได้





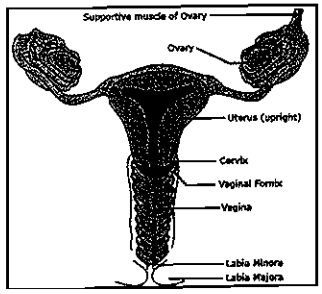


รุนแรงของโรคในรายที่โรคลุกลามมาก อาจต้องทุกข์ทรมาน และถึงแก่ชีวิตในปัจจุบันเป็นที่แน่ชัดว่า การติดเชื้อไวรัสฮิวแมนแปปิลโลมา (human papillomavirus หรือ HPV) หรือเชื้อไวรัสหงอนไก่ซึ่งสามารถติดต่อกันทางเพศสัมพันธ์เป็นสาเหตุหลักที่ทำให้เกิดมะเร็งปากมดลูก

แม้ว่าไวรัสหงอนไก่เป็นไวรัสที่ติดต่อทางเพศสัมพันธ์ในมนุษย์ ไวรัสหงอนไก่หรือไวรัสเอชพีวีชนิด 16 และ 18 เป็นต้นเหตุมะเร็งปากมดลูกถึงร้อยละ 70 และเป็นมะเร็งชนิดที่คร่าชีวิตผู้หญิงประมาณ 3 แสนคนทั่วโลกต่อปี ซึ่งรวมถึงประมาณ 4 พันคนในสหรัฐ ขณะที่สถิติของไทยในปี พ.ศ. 2539 มีผู้ป่วยมะเร็งปากมดลูก 6,268 ราย และปี พ.ศ.2549 มีผู้เสียชีวิตถึง 1,632 ราย ขณะเดียวกันก็ได้รับข่าวดีจากบริษัทยาต่างประเทศที่ได้ประกาศผลสำเร็จในการทดสอบวัคซีนในมนุษย์กว่า 10,000 คน ซึ่งสามารถป้องกันการเกิดมะเร็งปากมดลูกได้ถึง 100% และผลทดลองกับคนไทยต้องรอการสรุปและการติดตาม

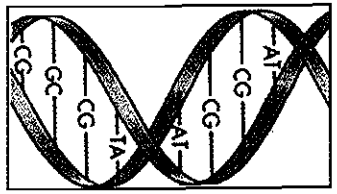
เริ่มแรก จากการตรวจคัดกรองมะเร็งปากมดลูก เช่น วิธีแป๊บสเมียร์ (Pap smear) อย่างไรก็ตาม การตรวจแป๊บสเมียร์ยังไม่สามารถครอบคลุมทุกพื้นที่ในประเทศไทยได้ดังนั้น ผู้ป่วยส่วนใหญ่ที่มาพบแพทย์จึงเป็นผู้ที่เป็นมะเร็งปากมดลูกระยะลุกลามและมีอาการมากแล้ว ทำให้การรักษายุ่งยากและสิ้นเปลืองค่าใช้จ่ายเพิ่มขึ้น การรักษาอาจจะหายหรือไม่ขึ้นอยู่กับความ

แพทยสภาได้ให้ข้อมูลเพิ่มเติมว่า วัคซีนมะเร็งปากมดลูกมีประสิทธิภาพในการป้องกันการติดเชื้อเอชพีวี 16 และ 18 ซึ่งเป็นไวรัสตัวการก่อโรคมะเร็งปากมดลูกที่สำคัญและจะสามารถช่วยแก้ปัญหาโรคมะเร็งอันดับหนึ่งที่คร่าชีวิตผู้หญิงไทยได้ ขณะนี้ยังอยู่ระหว่างการประเมินความคุ้มทุนที่จะประกาศให้เป็นแผนรณรงค์ฉีดภูมิคุ้มกันทั่วประเทศต่อไป



ความสำเร็จในการทดลองวัคซีนมะเร็งปากมดลูก นับเป็นข่าวดีสำหรับสตรีนับ 500,000 รายทั่วโลกที่ป่วยเป็นมะเร็งชนิดนี้ โดยในแต่ละปีจะมีสตรีที่เสียชีวิตด้วยมะเร็งปากมดลูกถึง 300,000 ราย แม้ว่าวัคซีนดังกล่าวสามารถป้องกันการติดเชื้อไวรัสเอชพีวีชนิด 16 และ 18 ได้ถึง 100% ด้วย การฉีดวัคซีน 3 โดส โดยใช้ระยะเวลาการติดตามผลนาน 24 เดือนแต่การเกิดมะเร็งปากมดลูกไม่ได้มีสาเหตุจากการติดเชื้อไวรัสเอชพีวีทั้งหมด นอกจากนี้สตรีที่ป่วยเป็นมะเร็งปากมดลูกอีกประมาณร้อยละ 30 เกิดจากสาเหตุอื่นนอกเหนือจากการติดเชื้อไวรัสเอชพีวีชนิด 16 และ 18 ดังนั้น ปัจจัยเสี่ยงต่อการเกิดมะเร็งปากมดลูกจึงยังรอการค้นหาเพื่อนำมาซึ่งการป้องกันที่มีประสิทธิภาพเพื่อสุขภาพที่ดีของสตรีต่อไป เนื่องจากการติดเชื้อไวรัสหงอนไก่ไม่ได้ทำให้เกิดมะเร็งปากมดลูกทุกราย ซึ่งแสดงให้เห็นว่าน่าจะมีปัจจัยเสี่ยงอื่นอีกที่เป็นสาเหตุทำให้เกิดความผิดปกติของเซลล์ปากมดลูก เช่น ลักษณะทางพันธุกรรมและพฤติกรรมเสี่ยง

ดังนั้นการทราบปัจจัยเสี่ยงที่เป็นสาเหตุของการเกิดมะเร็งปากมดลูก



จึงช่วยในการวางแผนการป้องกันรวมถึงเป็นแนวทางในการวางแผนการรักษาเซลล์ปากมดลูกที่เริ่มผิดปกติ จนกระทั่งเป็นมะเร็งปากมดลูกที่ยังไม่ลุกลามของประชากรในภาคตะวันออกเฉียงเหนือนี้ได้

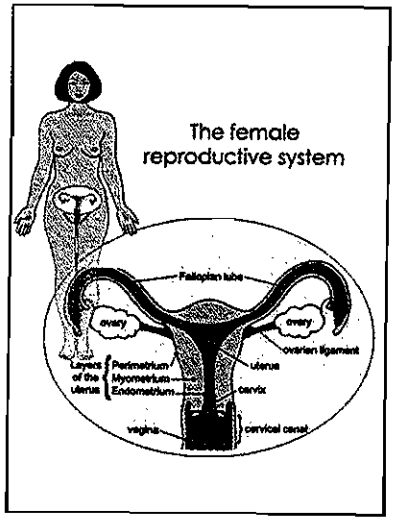
จากการศึกษาก่อนหน้านี้และพบว่าปัจจัยเสี่ยงด้านพฤติกรรมทางเพศและสูบบุหรี่ เช่น การมีเพศสัมพันธ์เมื่ออายุน้อยกว่า 17 ปี การมีคู่นอนมากกว่า 1 คน และการได้รับบุหรี่ทางอ้อม (passive smoking) จากคนใกล้ชิด (สามี) เป็นปัจจัยเสี่ยงสูงที่มีนัยสำคัญต่อการเกิดมะเร็งปากมดลูกของสตรีที่อาศัยอยู่ในพื้นที่ (Settheethamlshida et al., 2004a) อย่างไรก็ตามสตรีที่ได้รับบุหรี่ทางอ้อมจะมีโอกาสเกิดมะเร็งปากมดลูกรวมทั้งติดเชื้อ HPV ได้ง่ายกว่าสตรีที่ไม่ได้รับบุหรี่แต่การได้รับบุหรี่ไม่ได้ทำให้เกิดมะเร็งปากมดลูกและ/หรือติดเชื้อ HPV ในสตรีทุกราย (Setthee-tham-Ishida et al., 2004a, 2004b)

แม้การศึกษาด้านอณูระบาดวิทยาพันธุกรรม ซึ่งเป็นการศึกษาปัจจัยด้านบุคคลที่อาจจะมีผลเกี่ยวข้องกับการเกิดมะเร็ง จะมีความซับซ้อนสูงและทำการศึกษาได้ ค่อนข้างยากจากความก้าวหน้าด้านเทคโนโลยีที่ใช้ในการศึกษาวิจัยในปัจจุบัน การศึกษาด้วย

การวิเคราะห์ความหลากหลายของจีน (genetic polymorphism) เป็นวิถีใหม่ที่ทำให้มีโอกาสในการศึกษาและเข้าใจความสัมพันธ์ของอณูระบาดวิทยาพันธุกรรมกับการเกิดมะเร็งได้มากยิ่งขึ้น ข้อมูลที่แตกต่างกันของตำแหน่งต่าง ๆ บน จีนอาจจะมีผลต่อผู้สัมผัสสารก่อมะเร็งแตกต่างกัน จากความรู้นี้จะช่วยให้เข้าใจถึงปัจจัยเสี่ยงที่มีในแต่ละบุคคลได้จำเพาะมากขึ้นที่สามารถให้เป็นเครื่องหมายทางพันธุกรรมที่จะช่วยให้สามารถหลีกเลี่ยงป้องกันและรักษาการเกิดมะเร็งได้อย่างเหมาะสม (Yoshimura et al., 2003)

ในร่างกายมนุษย์จะประกอบด้วยกระบวนการการทำงานที่ซับซ้อนและเกี่ยวข้องกับสารก่อมะเร็งหลายชนิดโดยมี cytochrome P450 (CYP) เป็นเอ็นซัยม์ระยะแรก (phase I enzymes) ที่มีบทบาทเกี่ยวข้องกับเมแทบอลิซึมของสารก่อมะเร็งในร่างกายในการทำลาย หรือเพิ่มฤทธิ์ของสารก่อมะเร็งที่เกิดจากสารอัลฟาทอกซินบี 1 สารไนโตรซามิน (nitrosamines เช่น NNK, NNAL และ NDEA) รวมทั้งสารก่อมะเร็งในควันบุหรี่ที่มีหลายชนิด ซึ่งสารเหล่านี้ สามารถเปลี่ยนรูปเป็นสารก่อมะเร็งได้ (Aoya maet al., 1990; Crespi et al., 1991; Yamazaki et al., 1992; and Crespi et al., 1990) สารก่อมะเร็งนี้สามารถเข้ารวมตัวกับ DNA หรือเข้าทำลาย DNA แล้วทำให้มีการเปลี่ยนแปลงของเซลล์ที่ปกติในร่างกายและพัฒนากลายเป็น เซลล์มะเร็ง ในที่สุด P450 enzymes มีหลายชนิด เนื่องจากลักษณะทาง พันธุกรรมของ metabolizing enzyme แต่ละชนิด มีความหลากหลายทำให้การทำหน้าที่ของเอ็นซัยม์นี้มีความแตกต่างกัน รวมทั้งชนิดของเอ็นซัยม์นี้จะมีความแตกต่างกันในปัจเจกบุคคลด้วยซึ่งอาจมีความสัมพันธ์กับความเสี่ยงต่อการเกิดโรคมะเร็งปากมดลูกที่แตกต่างกันด้วย

CYP1A1 เป็น phase I enzyme ที่สำคัญใน metabolic activation ของ polycyclic hydrocarbons ซึ่งเป็นสารก่อมะเร็งที่พบในบุหรี่และสามารถตรวจพบสารนี้ได้ในน้ำอสุจิ (Coker et al, 2002) ความหลากหลายทางพันธุกรรมของ CYP1A1 มีหลายลักษณะ เช่น การเปลี่ยนจาก C เป็น T ใน noncoding 3'-flanking region มีผลต่อ MspI restriction site (MspIpolymorphism) เป็นลักษณะทางพันธุกรรมสำคัญอันหนึ่งที่ทำหน้าที่ควบคุมการแสดงออกของจีน CYP1A1 (Goodman et al, 2001; Sugawara et al, 2003) และเชื่อว่ามีความสัมพันธ์กับความเสี่ยงต่อการเกิดมะเร็งปากมดลูก (Good man et al, 2001) และโรคมะเร็งอื่นหลายชนิด เช่น มะเร็งตับ(Agundez, 2004) มะเร็งปอด (Ishibe et al, 1997) และมะเร็งช่องปาก (Park et al, 1997) เป็นต้น นอกจากนี้ metabolizing gene ชนิดอื่นอาจจะมีความสัมพันธ์กับการเกิดมะเร็งปากมดลูก มีรายงานการศึกษาพบว่า CYP 2D6 (PM, HET, EM) อาจจะมีความสัมพันธ์กับการเกิดมะเร็งปากมดลูก ทั้งในระยะก่อนลุกลามและระยะลุกลามด้วย (Warwick et al 1994a, 1994b) นอกจากนี้ CYP2A6 เป็นจีนชนิดหนึ่งที่เกี่ยวข้องกับเมตาบอลิซึมของนิโคติน (nicotine) ในบุหรี่และยาบางชนิด พบว่าจะมีบทบาทหลักที่ตับรวมทั้งพบในระดับต่ำที่อวัยวะอื่น ๆ ของร่างกายด้วย อย่างไรก็ตามยังมีความสับสนในด้านข้อมูลที่พบความแตกต่างของ P450 enzymes ต่อการเกิดมะเร็ง ปากมดลูกกันอยู่ (Sierra Torres et al, 2003, Warwick et al 1994a, 1994b, Good man et al, 2001, Kim et al, 2000)



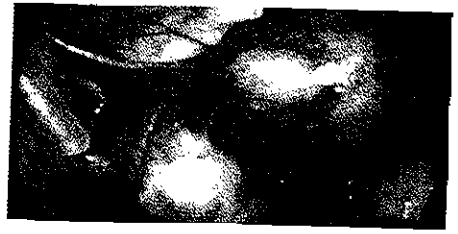
ระบบการซ่อมแซมดีเอ็นเอมีความจำเป็นต่อการดำรงและคงอยู่ของสารพันธุกรรม มีความสำคัญต่อการป้องกันการถูกทำลายและการกลายพันธุ์ของสารพันธุกรรมที่เป็นสาเหตุของการนำไปสู่การเกิดมะเร็ง ความเสื่อมถอยของระบบประสาทและการชราภาพ (Mathonetto et al.2003) จากการศึกษาความหลากหลายของจีนในส่วน functional polymorphisms ของจีน APEX1 XRCC1 ERCC4 และ ERCC2 (Shen et al. 1998), hMSH2 (Liu et al. 1995) รวมทั้งจีน hMLH1 และ hMSH3 (Benachenhou et al. 1998) ได้ข้อสรุปว่าประสิทธิภาพของระบบการซ่อมแซมดีเอ็นเอในแต่ละบุคคลจะมีความแตกต่างกัน (Crompton and Ozsahin 1997; Mohrenweiser and Jones 1998; Pero et al. 1983; Pero et al. 1989) ในมนุษย์สามารถแบ่งจีนที่เกี่ยวข้องกับการซ่อมแซมดีเอ็นเอตามวิถีการทำงานได้ 5 กลุ่มใหญ่ได้แก่ (Wood et al., 2001; Yu et al., 1999) 1) Direct repair pathway 2) Baseexcision repair (BER) pathway 3) Nucleotideexcision repair (NER) pathway 4) Doublestrand break (DSB) repair pathway. 5) Mismatched repair (MMR) pathway

XRCC1 protein จะทำงานร่วมกับ DNA ligase III, polymerase,poly (ADP-ribose) polymerase (Caldecott

"แพทยสภาได้ให้ข้อมูลเพิ่มเติมว่าวัคซีนมะเร็งปากมดลูกมีประสิทธิภาพในการป้องกันการติดเชื้อเอชพีวี 16 และ 18 ซึ่งเป็นไวรัสตัวการก่อโรคมะเร็งปากมดลูกที่สำคัญ และจะสามารถช่วยแก้ปัญหาโรคมะเร็งอันดับหนึ่งที่คร่าชีวิตผู้หญิงไทยได้ ขณะนี้ยังอยู่ระหว่างการประเมินความคุ้มทุนที่จะประกาศให้เป็นแผนรณรงค์ฉีดภูมิคุ้มกันทั่วประเทศต่อไป"

et al. 1996) และ apurinic endo nuclease APEX1 (Demple et al. 1991) ส่วนโปรตีน hMLH1 และ hMSH3 จะช่วยซ่อมแซมความผิดพลาดของดีเอ็นเอ (Kolodner 1996) ส่วนโปรตีน ERCC4 จะมีผลร่วมกับ nuclease จับกับ 5 ends ของดีเอ็นเอ (Bessho et al. 1997) ขณะที่โปรตีน ERCC2 ประกอบด้วย 5-3 ATP-dependent helicase activity (Sung et al. 1993) ที่เกี่ยวข้องกับ TFIIH protein complex ซึ่งมีความจำเป็นสำหรับการคัดลอกจีนและการซ่อมแซมดีเอ็นเอ (Leh mann 1995) กรดอมิโนที่ถูกแทนที่ด้วยโปรตีนเหล่านี้ อาจส่งผลกระทบต่อประสิทธิภาพในการซ่อมแซมดีเอ็นเอและต่อการปรับเปลี่ยนความเสี่ยงในการพัฒนาเป็นมะเร็งในแต่ละบุคคล (Spitz et al. 2003) มีการศึกษาพบว่าความหลาก หลายของจีนที่เกี่ยวข้องกับการซ่อมแซมดีเอ็นเอมีความสัมพันธ์กับการเกิดมะเร็งหลายชนิด และสามารถส่งผลต่อการตอบ สนองต่อการรักษาด้วย

นอกจากนี้ ยังมีจีนอีกหลายชนิดที่อาจเกี่ยวข้องกับการเกิดมะเร็งปากมดลูก เช่น จีนที่เกี่ยวข้องกับระบบภูมิคุ้มกันของร่างกาย โดยเฉพาะ lymphokines ที่ทำหน้าที่สำคัญในการป้องกันและกำจัดเชื้อโรคที่เข้าสู่ร่างกายที่จะส่งผลต่อการพัฒนาการเกิดโรคที่แตกต่างกัน



เนื่องจากลักษณะของกรรมพันธุ์มีความแตกต่างกันในแต่ละเชื้อชาติและยังขาดข้อมูลด้านความสัมพันธ์ของการเกิดมะเร็งกับกลุ่มจีนที่เสี่ยงต่อการเกิดมะเร็งที่แน่ชัด ดังนั้นความรู้ในด้านความถี่ของจีนอย่าง เช่น การแปรเปลี่ยนของจีนในประชากรจึงมีความจำเป็นต่อการศึกษาระบาดวิทยาของจีนและการศึกษาด้านเภสัชพันธุกรรมซึ่งสามารถใช้เป็นเครื่องหมายด้านกรรมพันธุ์ที่จะบ่งชี้ถึงเป็นสาเหตุและปัจจัยด้านพันธุกรรมที่มีผลต่อการเกิดมะเร็ง อันจะช่วยในการวินิจฉัยให้การป้องกันการแนะนำและการรักษาที่ถูกต้องเหมาะสม สามารถลดอุบัติการณ์การเกิดมะเร็งปากมดลูก ลดการสูญเสียทรัพยากรบุคคลลดการสูญเสียด้านเศรษฐกิจ รวมถึงลดปัญหาครอบครัวและสังคมของไทยในภาพรวม

(บทความนี้ได้รับการสนับสนุนจากโครงการวิจัย ประเภทกลุ่มวิจัย คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น)

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การผลิตและพัฒนาผลิตภัณฑ์อาหารว่าง จากข้าวเม่า

"ข้าวเม่า" ผลิตภัณฑ์อาหารในท้องถิ่นที่แปรรูปเป็นผลิตภัณฑ์อาหารว่างได้หลายชนิด ถ่ายทอดความรู้จากภูมิปัญญาท้องถิ่นมาหลายชั่วอายุ เนื่องจากลักษณะเฉพาะตัวของข้าวเม่าคือความหอมและสีเขียวธรรมชาติของเมล็ดข้าวทำให้ข้าวเม่าเป็นที่ยอมรับของผู้บริโภคทั่วไป อย่างไรก็ตาม ผลิตภัณฑ์เหล่านี้ยังคงมีปัญหาในการเก็บรักษา ผลิตภัณฑ์หลายชนิดจากข้าวเม่ามีความชื้นสูง จึงเสียง่ายและยังไม่มีการพัฒนาผลิตภัณฑ์แปรรูปอาหารชนิดใหม่ที่มีความแปลกใหม่ รวมทั้งให้มีคุณภาพและมาตรฐาน ซึ่งเป็นที่ยอมรับในระดับสากลและน่าสนใจต่อผู้ซื้อในวงกว้าง

รศ.ดร.วิเชียร วรพุทธพร จากภาควิชาเทคโนโลยีอาหาร คณะเทคโนโลยี ได้ศึกษาวิจัยเชิงปฏิบัติการแบบมีส่วนร่วมในการพัฒนาผลิตภัณฑ์ข้าวเม่ามาเป็นผลิตภัณฑ์อาหารเช้า **"ซีเรียลข้าวเม่า"** ขึ้น เพื่อให้เป็นสินค้ากลุ่มอาหารเช้าจากธัญพืชให้มีคุณภาพและมาตรฐานเป็นที่ยอมรับของผู้บริโภคทดแทนการนำเข้าธัญพืชบางส่วนจากต่างประเทศได้และคัดเลือกสูตรผลิตภัณฑ์ข้าวเม่าอาหารเช้าเพื่อนำไปเผยแพร่ เพื่อการจำหน่าย

NRCT-JSPS JOINT SEMINAR 2007

on

Infectious Diseases and Related Areas

Organized by

Mahidol University & The University of Tokyo

November 30, 2007

Mahidol University

PROGRAM
NRCT -JSPS JOINT SEMINAR 2007

Mahidol University, Salaya Campus

November 30, 2007

8.30-9.00 **REGISTRATION**
(Room 1210, Mahidol University International College)

9.00-9.20 **OPENING CEREMONY**

Welcome Address by **Prof. Dr. Ahnond Bunyaratavej**
Secretary General,
National Research Council of Thailand
Prof. Dr. Katsushi Tokunaga
NRCT- JSPS Core University Co-ordinat
The University of Tokyo
Opening Address by **Prof. Dr. Pornchai Matangkasombu**
President of Mahidol University

GROUP PHOTO SESSION

9.20-9.40 **BREAK**

9.40-12.00 **GENETICS & DISEASES**
Chairperson: Prof. Dr. Srisin Khusmith
Vice President for Research, Mahidol University

- Genome-Wide Search for Susceptibility Genes to Complex Diseases
  Prof. Dr. Katsushi Tokunaga
  Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, ***Japan***

- Linkage Analysis for Identification of Susceptibility Gene in Infectious Diseases
  Dr. Surakameth Mahasirimongkol
  Center for International Cooperation, Department of Med Science, Ministry of Public Health, ***Thailand***

- Purification and Characterization of Human Peripher Blood Regulatory T Cells Using FACSAria
  Dr. Ryo Takahashi
  *Division of Flow Cytometry, Kyorin University School of Medicine, **Japan***

- Evaluation of the Immunochromatography Test for Rapid Detection of Norovirus Antigen in Stool Sampl
  Dr. Pattara Khamrin
  *Department of Developmental Medical Sciences, Institute International Health, Graduate School of Medicine, The University of Tokyo, **Japan***

14.20-14.30 **BREAK**

14:30-15.30 **ETHNO-EPIDEMIOLOGY OF VIRUS ASSOCIATED CANCERS**

Chairperson: Prof. Dr. Kiyoshi Kita
Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo

- Ethnoepidemiology of Virus Associated Cancers in Thailand
  Prof. Dr. Takafumi Ishida
  *Human Biology & Genetics, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, **Japan***

- Genetic Polymorphisms and Cancer Risk
  Dr. Danai Tiwawech
  *Research Division, National Cancer Institute, **Thailand***

- Cervical Cancer in Northeastern Thailand
  Assoc. Prof. Dr. Wannapa Settheetham-Ishida
  *Department of Physiology, Faculty of Medicine, Khon Ka University, **Thailand***

Cervical cancer in Northeastern Thailand

Wannapa Settheetham-Ishida

Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

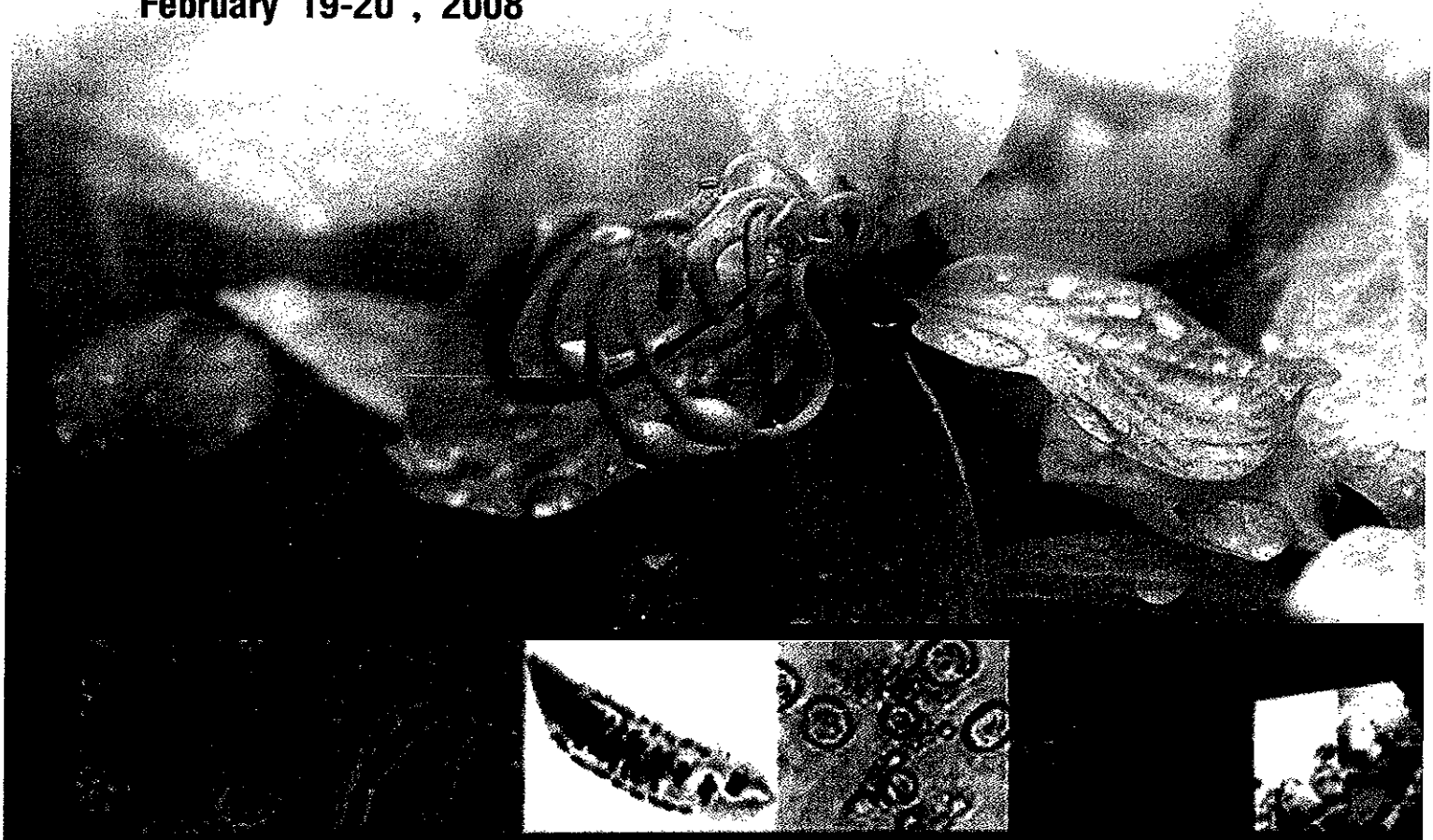
Cervical cancer remains the most common cancer among Thai women ev
though its incidence has decreased according to the age-standardized ra
Contrariwise, other data suggest the incidence rate of this cancer gradua
increased in Northeast Thailand between 1992 and 2000. HPV infection is
major cause of cervical cancer and has been confirmed as a critical risk factor
the development of cancer in this region. The prevalence of HPV-16 infection w
prominent, followed by HPV-18. The number of sexual partners and passi
smoking history were identified as risks for HPV infection. As for passi
smoking, tobacco specific carcinogens/mutagens may exist in the cervical muc
through smoking and/or in the semen of tobacco smoking partners. Cervic
exposure to such carcinogens can reduce cervical immunity resulting in
persistence of HPV infection and also generating DNA lesions. It is hypothesiz
that certain genetic backgrounds, such as chemical metabolizing enzyme gen
may play roles in the development of this cancer.

Proceedings of

The Asia-Africa International Symposium of JSPS
Asia and Africa Science Platform Program and
The Fourth LiverCare Center Symposium

Infection-Immunity and Cancer

Charoen Thani Princess Hotel, Khon Kaen, Thailand
February 19-20th, 2008



Organized by:
The Liver Fluke and Cholangiocarinoma Research Center and
Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Thailand
Graduate School of Medical Sciences, Kumamoto University, Japan

P-16

Glutathione S-Transferases (*GSTM1* and *GSTT1*) and Smoking Habit in Cervical Cancer in Northeast Thailand

Wannapa Settheetham-Ishida[1], Pissamai Yuenyao[2], Wichittra Tassaneeyakul[3], Churairat Kularbkaew[4], Dariwan Settheetham[5], Takafumi Ishida[6]

[1]Department of Physiology, [2]Department of Obstetrics and Gynecology, [3]Department of Pharmacology, [4]Department of Pathology, Faculty of Medicine, [5]Department of Environmental Health, Faculty of Public Health, Khon Kaen University, Khon Kaen 40002, Thailand [6]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan

Genotypes of glutathione S-transferases (*GSTM1* and *GSTT1*) and cervical cancer were studied with special reference to the smoking habit in Northeastern Thailand. An overall prevalence of *GSTM1*-null genotype in the controls and the cervical cancer patients was 59.6% and 60.0%, respectively and of *GSTT1*-null genotype in the control and the cervical cancer patients was 40.4% and 46.7%, respectively. There were not statistical differences in the genotype either of *GSTM1* or *GSTT1* between the cases and the controls ($p>0.05$). Although the combination of *GSTM1*-null and *GSTT1*-null genotype showed a trend to increase the risk of cervical cancer with adjusted OR =2.72, it was not significant ($p = 0.10$). In relation with smoking habit, however, interaction between *GSTM1* or *GSTT1* showed that *GSTM1*-null genotype had higher risks for cervical cancer development (2.7-fold; $p=0.02$) in smokers. In conclusion, a lack of overall association between cervical cancer development and *GSTM1* and *GSTT1* genotypes was observed; however, a higher risk for the cervical cancer in the smokers with null-genotype was confirmed.

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CONTENTS

ORAL PRESENTATIONS

HPV AND BLADDER CANCER: A NEW CHALLENGE FOR THE UROLOGICAL RESEARCH

Tommaso Cai[1], Sandra Mazzoli[2], Riccardo Bartoletti[1]
[1]*Department of Urology, University of Florence, Bagno a Ripoli (Florence), Italy*
[2]*STDs Centre, Santa Maria Annunziata Hospital, Bagno a Ripoli (Florence), Italy*

Background: Infections with high-risk HPV types (HR-HPV), such as 16, 18 and 33, have been demonstrated in a high percentage of patients with several cancers. Moreover, HR-HPV infection has also been confirmed in urothelial cell carcinoma (UC) of the urinary bladder.

Objectives: To establish the pathogenetic role of HR-HPV in UC development and progression.

Materials and Methods: A series of 78 patients affected by histopathologically demonstrated UC were enrolled in the present study. From all patients, a sample of morning spontaneous voided urine was collected by using a sterile method and before surgery, in order to evaluate the presence of HR-HPV-DNA. The DNA extraction and purification from all biological materials was performed by DNeasy® Tissue Kit by QIAGEN Spa, Italy. The presence of genital HR-HPV-DNA was investigated by Alpha Watch HPV, Alphagenic-Diaco-Biotechnology, Trieste, Italy. Moreover, the presence of HR-HPV-DNA was evaluated both in urine and in tumour tissues obtained from surgery. 59 patients affected by bladder outlet obstruction (BOO) due to benign prostatic hyperplasia (BPH) and who had undergone TUR-P were considered as a control group.

Results: The presence of HR-HPV-DNA was reported in 27 out of 78 (34.6%) tumour samples and in 6 out of 59 (10.1%) specimens from TUR-P, with a statistically significant difference (p=0.003). On the other hand, the presence of high-risk HPV-DNA in urine samples was 36 out of 78 (46.1%) obtained from UC patients while 8 out of 59 (13.5%) from BPH patients (p=0.008). These data, even if they were to be confirmed by studies with a greater number of patients, require further assessment.

Conclusions: The role of HR-HPV in bladder carcinogenesis is still debatable, but the present data suggest a potential role of HR-HPV in bladder cancer development and progression, that should be taken into consideration in everyday clinical urological practice.

HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL ABNORMALITY IN NORTHEAST THAILAND

Wannapa Settheetham[1], Pissamai Yuenyao[2], Churairat Kularbkaew[3], Takafumi Ishida[4]
[1]*Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
[2]*Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
[3]*Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
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Materials and Methods: Case-control studies on human papillomavirus (HPV) infection were done in patients with cervical squamous intraepithelial lesions (SIL) and squamous cell cervical cancer (SCCA) in Northeastern Thailand.

Results: Prevalence of high-risk group of HPV (-16,-18,-31,-33,-35,-52b, and -58) infection was 13.0–18.1% in controls, 32.6% in low SIL (LSIL), 80.0% in high SIL (HSIL) and 86.7% in SCCA. HPV infection significantly increased risk for overall SIL 6.8-fold ($p<0.001$), and also increased the risk for transition from LSIL to HSIL 8.3-fold ($p<0.001$) resulting in the risk for HSIL as high as 18.1-fold ($p<0.001$). High risk HPV infection was also associated with the risk for cervical cancer 43.5-fold ($p<0.00001$). Among HPV positive patients, HPV-16 infection was the commonest (50%) in SIL and increased the risk for HSIL (OR=53.8; $p<0.001$). In the SCCA patients, HPV-16 was also prominent (70.5%) followed by HPV-18 (23.1%); however, statistical difference in the subtype distribution was not observed in between the SCCA and the control.

Conclusions: HPV-16 is a critical risk factor for LSIL to HSIL transition as well as cervical cancer development. In addition, as suggested by our previous study, smoking is associated with the risk for cervical cancer development, prevention of not only HPV infection causing cervical abnormalities but also smoking enhancing HPV infection and cervical hyperplasia should be emphasized in the public health scheme and education.

PROGNOSTICS RELEVANCE OF THE DETECTION OF HPV HIGH RISK DNA TYPES 16, 18 & 45 USING THE QIAGEN HPV-16/18/45 PROBE SET IN HPV HIGH RISK POSITIVE SPECIMENS: FIRST RESULTS

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Background: Although today HPV-infection is accepted to be one of the major risk factors and additionally is fairly common amongst younger women, generally the related abnormal and precancerous cervical lesions are successfully suppressed by the T-cell system. Yet, persistent infections with high risk HPV DNA types are associated with the development of cervical intraepithelial neoplasia (CIN) and might cause a progression to invasive cervical cancer.

Objectives: Cervical smears that were positive with the HC2 high risk test were retested with the Probe Set that detects the high risk HPV types 16, 18 and 45. Cytological results will be correlated with histology. Does the Probes Set offer a chance to identify relevant clinical infections?

Materials and Methods: Our material originates from 463 women. The study started in October 2007. It was ensured that all of the collected smears were positive for HR HPV-DNA by the HC-2 test. Smears were evaluated according to the Munich nomenclature. A conization was performed if clinically indicated. Follow up will be conducted according the German gynecological guidelines for two years.

Results: From the initial 463 smears 226 (48.8%) were abnormal (>PAP II). The rest demonstrated a normal cytology. 291 (65.8 %) of the high risk infections have been tested positive with the Probe Set, 118 (40.6 %) of them were morphologically inconspicuous and 173 (59.4 %) demonstrated signs of cervical lesions. Within this group two cases of CIS and one case of cervical cancer were detected.

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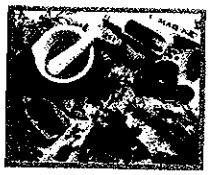

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Molecular epidemiology of DNA repair gene and cervical cancer susceptibility

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Introduction: DNA repair is essential to maintain cellular functions and homeostasis. However, the repair capacity can be altered based on DNA sequence variations in DNA repair genes and may cause cancer susceptibility.

Objective: This study aimed to investigate the association of genetic polymorphism in DNA repair genes and squamous cells cervical carcinoma (SCCA) among women in Northeast Thailand.

Method: Volunteers (n=111) with SCCA and age-matched healthy controls (n=117) were recruited at Srinagarind Hospital, Khon Kaen University. Polymorphism in DNA repair genes, XRCC1 Arg399Gln in exon 10 (G-->A), XRCC1 Arg194Trp in exon 6 (C-->T) and XRCC3 Thr241Met in exon 7 (C-->T) were studied using PCR-RFLP. The same interviewers conducted a questionnaire. The Ethics Committee at Khon Kaen University approved the study protocols then each subject gave written informed consent before being enrolled.

Results: Prevalence of the XRCC1 Arg399Gln of GG, GA, AA in the SCCA patients was 59.46, 36.94, 3.60 % and in the controls was 58.97, 36.75, 4.27 %, respectively.The proportion of XRCC1 Arg194Trp, CC, CT, TT genotypes in the SCCA patients was 47.75, 44.14, 8.11% and in the controls was 54.70, 43.59, 1.71%, respectively.The proportion, XRCC 3 Thr241Met, CC, CT genotypes in the SCCA patients was 90.99, 9.01% and in the controls was 89.74, 10.26%, respectively. Both of the XRCC1 Arg399Gln and XRCC3 Thr241Met genotypes did not increase the risk for SCCA ($p>0.05$). Whereas homologus XRCC1 194Trp allele was observed at higher risk for SCCA with Odd ratio (OR) =1.8 (95%CI=1.28-2.54, p=0.02) and adjusted OR=5.677 (95%CI=1.16-27.80, p=0.03) in XRCC1 Arg194Trp. The distribution of variant genotype of XRCC1 and XRCC 3 did not show higher risk for SCCA significantly ($p>0.05$), in smoking status.

Conclusion: This study indicates that *XRCC* polymorphisms play important roles in modifying individual susceptibility to cervical cancer, and the cancer susceptibility may depend on the tumor. The direction of our coming studies must describe more detailed history of exposure to procarcinogens and carcinogens.

Key Words: DNA repair gene, cervical cancer susceptibility