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PERB11 (MIC) as a possible susceptibility gene for nasopharyngeal cancer development

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Background : *There have been many studies indicating that a gene located near HLA A2 and HLA B46 is a tumor susceptibility gene involved in nasopharyngeal cancer (NPC) development. PERB11 (MIC) is a candidate gene since it is linked to HLA B.*

Objective : *To investigate the association between PERB11 (MIC) and the probability of having NPC.*

Materials and Methods : *The frequencies of six alleles from a triplet repeat polymorphism of the transmembrane region of MICA (PERB11.1) were analyzed by PCR from 300 healthy blood donors' and 171 NPC patients' DNA samples. The relative risk was estimated by odds ratio to determine the allele association with NPC patients in comparison with normal controls.*

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Result : *The frequency of the A6 allele, but not others, was increased in the patient group, as compared with the control group (RR = 1.6, P < 0.05, OR = 1.60, 95 % CI = 0.98-2.63).*

Conclusion : *One particular allele (A6) of the PERB11 (MIC) gene presents at a higher frequency in NPC patients than in controls. This suggests a possible association of NPC development with the PERB11 gene.*

Key words : *PERB11 (MIC) gene, Polymorphism, Nasopharyngeal cancer.*

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ปัญหาของการทำวิจัย : *มีการศึกษาพบว่ายีนที่อยู่บริเวณ HLA A2 และ HLA B46 เป็นยีนที่ส่งเสริมให้เกิดมะเร็งชนิดนี้ เนื่องจากยีน PERB11 (MIC) เป็นยีนที่อยู่ใกล้กันกับยีน HLA B ดังนั้น PERB11 (MIC) อาจจะเกี่ยวข้องกับการเกิดโรคมะเร็งโพรงหลังจมูก*

วัตถุประสงค์ : *เพื่อศึกษาหาความสัมพันธ์ระหว่างยีน PERB11 (MIC) กับการเกิดโรคมะเร็งโพรงหลังจมูก*

วัสดุและวิธีการวิจัย : *คณะผู้วิจัยได้ทำการศึกษาความสัมพันธ์ระหว่างยีน PERB11 (MIC) กับการเกิดโรคมะเร็งโพรงหลังจมูกโดยเปรียบเทียบระหว่างดีเอ็นเอจากเลือดคนปกติ 300 ราย และ 171 รายจากคนผู้ป่วยที่ได้รับการตรวจวินิจฉัยว่าเป็นโรคมะเร็งโพรงหลังจมูกโดยหาความสัมพันธ์ระหว่างความถี่ของอัลลีลทั้งหกของ triplet repeats ในส่วนของยีนที่สร้างโปรตีนอยู่ในเยื่อบุเซลล์ของยีน PERB11 (MIC) ในกลุ่มของผู้ป่วยกับคนปกติโดยดูถึง*

ผลการศึกษา : *พบว่าความถี่ของยีนเฉพาะอัลลีล A6 เพิ่มขึ้นในกลุ่มของผู้ป่วยเมื่อเปรียบเทียบกันกับกลุ่มคนปกติ (R.R.=1.6, P < 0.05, OR = 1.60, 95% CI = 0.98-2.63) แต่ไม่พบการเพิ่มขึ้นอย่างมีนัยสำคัญในอัลลีลอื่น ๆ ของยีน PERB11 (MIC)*

สรุป : *ข้อมูลของคณะผู้วิจัยพบว่ามีความถี่ของอัลลีล A6 เพิ่มขึ้นในกลุ่มผู้ป่วยที่เป็นโรคมะเร็งโพรงหลังจมูกเมื่อเปรียบเทียบกันกับกลุ่มคนปกติ แสดงให้เห็นถึงความเป็นไปได้ของความสัมพันธ์ระหว่างการเกิดโรคมะเร็งโพรงหลังจมูกกับยีน PERB11 (MIC)*

The etiology of nasopharyngeal carcinoma (NPC) has opened an interesting field of study concerning the interplay between genetic and environmental factors combined with Epstein – Barr virus (EBV) infection. NPC is rare among Caucasians, with incidence rates below 1 per 100,000 persons/ year. Among Chinese, with a high incidence rate (30 - 50 per 100,000/year) and Southeast Asians with an intermediate rate (3 -10 per 100,000 people/year), the possibility of a genetic contribution becomes apparent. There have been many reports indicating various human leukocyte antigen (HLA) alleles are associated with NPC. The link between HLA and NPC was first reported in Singapore [1] and this finding has subsequently been confirmed in several countries in Asia. [2] All have demonstrated the association with HLA-A2 and HLA-B46 (relative risk = 2.35). However, HLA-A2 subtyping studies have shown that it is unlikely to play a role in the EBV clearance hypothesis. In addition, in a causative association the risk would increase if both HLA-A2 and B46 were inherited on the same chromosome, i.e. haplotype. In contrast, this HLA haplotype in non-Chinese patients is not associated with NPC. These data suggest that HLA is not the susceptibility gene *per se*, but the NPC susceptibility gene locus is most likely to reside within the HLA region.

PERB11 (*MIC*), a major histocompatibility complex (MHC) class I chain-related gene, is located in the HLA region. *PERB11.1* (*MIC*A), an expressed *PERB11*, has recently been identified to be located near the HLA-B locus [3,4] and displays 6 distinct alleles of microsatellite polymorphism in the transmembrane (TM) region.[5,6] *MICA* is frequently expressed in epithelial tumors. Upon interaction between MICA and its receptor NKG2D, diverse innate anti-tumor NK cell and antigen-specific T-cell responses are triggered. [7] Therefore, *PERB11* (*MIC*) is a candidate tumor susceptibility gene.

In this study, we have investigated the correlation between short tandem repeat polymorphisms in the TM of *PERB11.1* (*MICA*) and NPC development in a total of 171 cases diagnosed with NPC and 300 controls. The hypothesis was that if one of the alleles was significantly increased in the patients, as compared with the control group, the *PERB11* (*MIC*) gene might be a NPC susceptibility gene.

Method

Samples and DNA extraction

Blood samples were obtained by venipuncture from 300 healthy blood donors (Thai Red-Cross Society) and 171 NPC patients (Chulalongkorn Hospital and National Cancer Institute). The diagnosis had been confirmed histologically and by the presence of EBV DNA in the tumors. DNA was extracted from blood leukocytes by methods previously described. [8]

PCR

For analysis of the microsatellite repeat polymorphism in the TM region of the *MICA* gene, PCR primers flanking the TM region were used (MICA5F,5'-CCTTTTTTTCAGGGAAAGTGC-3'; MICA5R, 5'-CCTTACCATCTCCAGAAACTGC-3'). [5] The PCR reactions were performed in a total volume of 10 μl using 50 ng of gemomic DNA, 200 μM each dNTP, 10 μM Tris-HCl (pH 8.4), 50 mM potassium chloride, 2.5 mM magnesium chloride, 0.5 units of *Thermus aquaticus* DNA polymerase (Perkin Elmer Cetus) and

0.1 μM of each primer. One of each primer pair was end labelled at 37°C for 1-2 h in a total volume of 10 μl containing 10 μM primer, 0.025 mCi [γ ^{32}P] ATP (Amersham Pharmacia Biotech) at 3000 Ci mmol^{-1}, 10 μM magnesium chloride, 5 mM DTT, 70 mM Tris-HCl (pH 7.6) and 10 units of T4 polynucleotide kinase (New England Biolabs). Without further separating of the unincorporated nucleotides, the kinase reaction was added to the PCR buffer mix. The PCR amplifications were performed as follows: initial denaturation at 94°C for 5 min, followed by 25 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 7 min.

Two microlitres of each reaction were mixed with 1 μl of formamide-loading buffer, heated at 95°C for 2 min, put on ice for 30 s and then loaded onto 6 % polyacrylamide/7 M urea gel. DNA fragments were size fractionated at 70 W until the tracking dye had covered the appropriate distance of the gel. After electrophoresis, the wet gel was transferred to filter paper (Whatman), wrapped with Saran wrap and exposed to a phosphorus screen; the bands were visualized on PhosphoImager using ImageQuaNT software (Molecular Dynamics).

Statistical Analysis

Gene frequencies were estimated by direct counting. The significance of the distrubution of alleles between NPC patients and normal controls were tested by chi-square (χ^2) method with continuity correction and Fisher's extra probability test (P value test).[9] Comparison between two groups was made with a 95 % confidence interval to estimate statistical significance.

Result and Discussion

To address the possibility that *MICA* is a susceptibility gene for NPC development, triplet repeat polymorphisms in the TM region of *PERB11.1* (*MICA*) were investigated in 171 cases diagnosed with NPC and 300 healthy blood donors as a control (Fig 1).

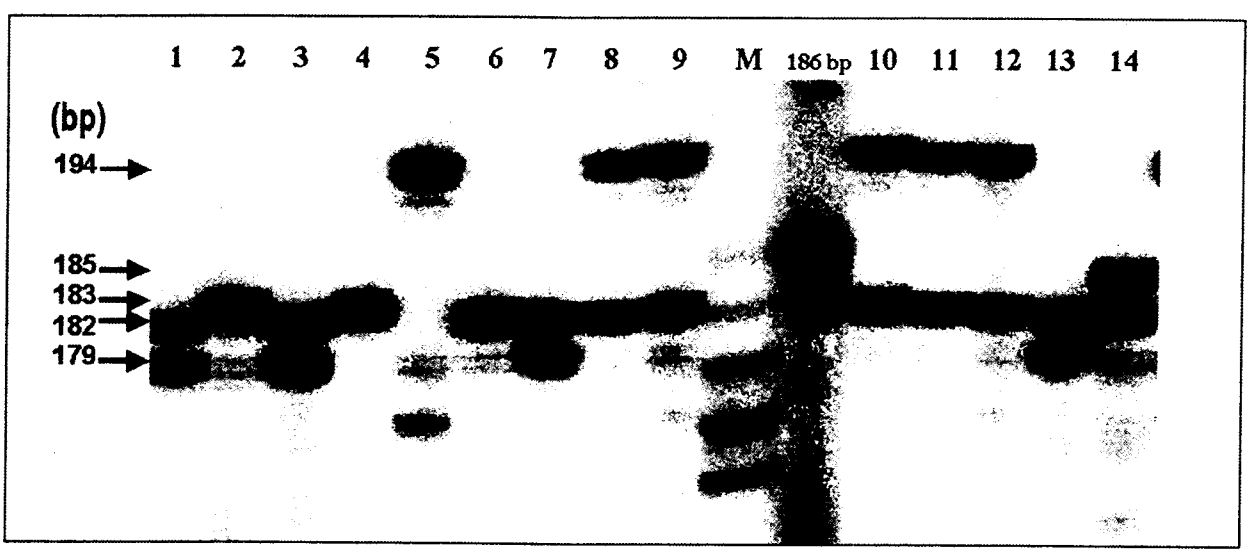

Figure 1. Microsatellite analysis of PCR-amplified products of triplet repeat polymorphism in the TM region of the PERB11.1 (MICA) gene. Cases 1-14: nasopharyngeal cancer patients. M, molecular marker.

PERB11 displays 6 distinct alleles of microsatellite polymorphism at the TM region. The frequency of A6 allele was significantly increased in the patient group, as compared with the control group (R.R.=1.6, $P < 0.05$, O.R. = 1.60, 95 % CI = 0.98 - 2.63) (Table 1) but none of the other alleles showed any significant association. These data suggest a possible important role for this allele in the development of NPC. Interestingly, A6 is the same allele found to be associated with Beh?et disease in the Japanese population. [5]

In conclusion, we have found that one particular allele (A6) of the microsatellite is present at a higher frequency in NPC patients than in controls. Yet, since the 95 % CI contains the nominator 1 the OR value dies not achieve significance, This may well be due to the small sample size employed in the present study. Since the TM polymorphism is not well correlated with polymorphisms of the extracellular domains, [10] it is essential to further evaluate this polymorphism to establish the relevance of this gene family in NPC development.

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Table 1. Gene frequencies of the microsatellite polymorphism in the TM region (exon 5) of the PERB11.1 (MICA) gene in nasopharyngeal carcinoma.

Microsatellite allele	Amplified product (bp)	Control (n = 600)	Patient (n = 342)	P value	R.R.	95% CI
A4	179	87	40			
A5	182	220	129			
A5.1	183	138	70			
A6	185	41	36	0.046	1.60	0.98 - 2.63
A9	194	114	67			

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