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Interferon - γ gene (+874) polymorphism in Thai Population

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Background : *Interferon (IFN) – γ plays a key role in the defense against viruses, intracellular pathogens and induction of inflammatory response. Polymorphisms of IFN–γ genes may effect gene transcription, causing individual variation in IFN–γ production. IFN–γ gene at +874 polymorphisms, which was associated with IFN-γ production, have also been reported associated with susceptibility and clinical severity of diseases in several studies. However, the results are varied between each ethnic group.*

Objective : *This study was to investigate the polymorphisms of IFN–γ gene at +874 in Thai population and compare the distribution between Thai population and other population.*

Design : *In vitro experimental study*

Method : *This study included 137 unrelated healthy Thai individuals. Polymorphisms at +874 within intron 1 of the IFN-g gene were identified using the PCR-sequence specific primer (SSP) method. Genotype and allele frequencies were compared between Thai and other population using Chi-square (χ^2) test.*

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Result : *The genotype frequencies in Thai population were 60.6 % for A/A; 33.6 % A/T; and 5.8 % T/T. Genotype frequencies of healthy Thai individuals were in Hardy-Weinberg equilibrium. There were significant differences in allele and genotype frequencies between Thai population with Italian (p=0.001 and p=0.00002, respectively) and Brazilian Caucasian (p=0.03 and p=0.002, respectively). In contrast, there were no significant differences in allele and genotype frequencies between Thai population with South African and Asian.*

Conclusion : *Distribution of IFN-γ gene (+874) polymorphism Thai population were significantly different when compared with Italians and Brazilian Caucasians. These differences seem to be the apparent influence of ethnicity.*

Keywords : *Interferon-γ gene polymorphisms, Thai population.*

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จีรวัฒน์ นาคขุนทด, พิมพเยาว์ สดใส, ณัฏฐิยา หิรัญกาญจน์. ความหลากหลายของยีนอินเตอร์เฟียรอนแกมม่าที่ตำแหน่ง +874 ในประชากรไทย. จุฬาลงกรณ์เวชสาร 2549 ต.ค; 50(10):685 - 94

บทนำ : *อินเตอร์เฟียรอนแกรมม่ามีบทบาทสำคัญในระบบภูมิคุ้มกันของร่างกายทั้งในด้านการต่อต้านไวรัสและจุลชีพภายในเซลล์ ตลอดจนกระตุ้นให้เกิดสภาวะการอักเสบ ความหลากหลายของยีนอินเตอร์เฟียรอนแกรมม่าอาจส่งผลต่อการ transcription และทำให้เกิดความแตกต่างในระดับการสร้างอินเตอร์เฟียรอนแกรมม่า มีรายงานหลายฉบับพบความสัมพันธ์ระหว่างความหลากหลายของยีนอินเตอร์เฟียรอนแกรมม่าที่ตำแหน่ง +874 (ซึ่งเป็นตำแหน่งที่สัมพันธ์กับระดับการสร้างอินเตอร์เฟียรอนแกรมม่า) กับความเสี่ยงในการเกิดโรคและความรุนแรงของโรค แต่อย่างไรก็ตามผลจากการศึกษาก็ยังคงมีความแตกต่างกันในแต่ละเชื้อชาติ*

วัตถุประสงค์ : *เพื่อศึกษาความหลากหลายของยีนอินเตอร์เฟียรอนแกรมม่าที่ตำแหน่ง +874 ในประชากรไทย และเปรียบเทียบความแตกต่างกับกลุ่มประชากรอื่น ๆ*

รูปแบบการศึกษา : *In vitro experimental study*

วิธีการศึกษา : *การศึกษานี้ได้รวบรวมประชากรไทยที่มีสุขภาพดี จำนวน 137 คน และใช้วิธี PCR- sequence specific primer (SSP) ในการศึกษาความหลากหลายของยีนอินเตอร์เฟียรอนแกรมม่าที่ตำแหน่ง +874 ที่อยู่ในส่วนอินตรอนที่ 1 จากนั้นนำความถี่ของ genotype และ allele ที่พบในประชากรไทยเปรียบเทียบกับประชากรอื่น ๆ โดยใช้ Chi-square (χ^2) test ในการเปรียบเทียบความแตกต่าง*

ผลการศึกษา : *ความถี่ของ genotype ที่พบในประชากรไทยมีดังนี้ พบ genotype แบบ A/A ทั้งหมด 60.6 % พบ genotype แบบ A/T ทั้งหมด 33.6 % และ พบ genotype แบบ T/T ทั้งหมด 5.8 % โดยความถี่ของ genotype อยู่ในสมดุลของ Hardy-Weinberg พบความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบความถี่ของ allele และ genotype ของชาวไทยกับชาวอิตาลี (p=0.001 และ p=0.00002 ตามลำดับ) และ Brazilian Caucasian (p=0.03 และ p=0.002 ตามลำดับ) แต่กลับไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับ South African และชาวเอเชียที่มีรายงานไว้ก่อนหน้านี้*

สรุป : *ความหลากหลายของยีนอินเตอร์เฟียรอนแกรมม่าที่ตำแหน่ง +874 ในประชากรไทยมีการกระจายที่แตกต่างอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกันชาวอิตาลี และ Brazilian Caucasians โดยการกระจายที่แตกต่างกันนี้น่าจะมีผลจากความแตกต่างของเชื้อชาติ*

คำสำคัญ : *ความหลากหลายของยีนอินเตอร์เฟียรอนแกรมม่า, ประชากรไทย*

Interferon (IFN)-γ (type II IFN) is a Th1 cytokine and plays a key role in modulating almost all immune responses, such as hematopoiesis, immune-mediated inflammatory response, anti-tumor, and defense against viruses and intracellular pathogens. IFN-γ is a biologically active non-covalently linked homodimer (MW18,000 polypeptides) secreted primarily by NK cells, Th1 $CD4^+$ T-cells and $CD8^+$ T-cells. In most cells, including professional antigen presenting cells, IFN-γ upregulates the expression of major histocompatibility complex (MHC) class I and II molecules. Many studies have reported that a different level of IFN-γ expression plays a significant role in the susceptibility and clinical severity of diseases.[1] In particular, previous study has suggested that polymorphism in IFN-γ gene is related to the protein secretion.

The gene for human IFN-γ is located at chromosome 12q24.1.[2] Two previous studies have described a variable length CA repeat sequence in the first intron of the human IFN-γ gene and showed that allele 2 is associated with high *in vitro* IFN-γ production.[2, 3] Furthermore, Pravica and co-workers reported an absolute correlation between this 12-CA-repeat allele (allele 2) and the presence of the T allele at a single nucleotide polymorphism (SNP) located at the +874 position (+874T/A) from the translation start site, coinciding with a putative NF-κB binding site that might be important in the induction of constitutively high IFN-γ production. Therefore, it has been suggested that the T to A polymorphism at +874 (at the 5' end of the CA repeat) directly influences the level of IFN-γ production associated with the CA microsatellite marker.[4] Several studies have reported the association between IFN-γ gene polymorphism at +874 with the susceptibility and clinical severity of disease, such as tuberculosis, breast cancer, chronic allograft nephropathy and *Helicobacter pylori* disease outcome.[5-8] However, the results are varied between each ethnic group. The aim of this study was to investigate the polymorphism at the +874 of IFN-γ gene in Thai population and compare the distribution between Thai population and other populations previously reported in the literatures.

Material and Method

Subjects

One hundred and thirty-seven unrelated healthy Thai individuals (100 females and 37 males; mean age ± SD = 23 ± 12.3 years), were recruited from healthy blood donors at the Thai Red Cross Society. These donors have Thai/Chinese ethnic.The study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University.

Genotyping methodology

Genomic DNA was extracted from buffy coat collected with EDTA as anticoagulant, using a salting out method.[9] Polymorphisms at +874 within intron 1 of the IFN-γ gene were identified using the PCR- sequence specific primer (SSP) method. All DNA were amplified with the use of the IFN-γ gene specific primers (forward, T allele; 5'-TTCTTACAACACAAAATCAAATCT-3', A allele; 5'-TTCTTACAACACAAAATCAAATCA-3' and reverse, 5'-TCAACAAAGCTGATACTCCA-3') described by Pravica and colleagues.[4] The reaction volume for the amplification reaction was 20 μl, containing 50 ng/μl genomic DNA, 0.1 μl of 5.0 Unit/μl Taq

polymerase (Promega), 2 μl of 10x PCR buffer (20mM Tris-HCl pH 8.0, 100 mM KCl), 1.2 μl of 25 mM $MgCl_2$, 0.4 μl of 10 mM dNTP and 1 μl (20 pmol) of each primer and 0.1 μl (20 pmol) of internal control primers. Internal control primers were used to check for successful PCR amplification (forward, 5'-GCCTTCCCAACCATTCCCTTA-3' and reverse, 5'-TCACGGATTTCTGTTGTGTTTC-3'). These primers amplify a human growth hormone sequence. Amplification was performed in Perkin Elmer/ GeneAmp PCR system 2400. The PCR protocol consisted of an initial denaturation at 95°C for 1 minutes, followed by 10 cycles of denaturation (95°C, 15 seconds), annealing (62°C, 50 seconds) and extension (72C, 40 seconds) and 20 cycles of denaturation (95°C, 20 seconds), annealing (56°C, 50 seconds) and extension (72°C, 50 seconds) final extension at 72°C for 7 minutes.[4] The positive results of IFN-γ gene and human growth hormone gene showed band of 261 and 428 bp fragment, respectively. Additionally, the PCR products were analyzed to confirm the results of IFN-γ genotyping by DNA sequencing. Specific primers for sequencing (forward, 5'- GCTGTCATAATAATATTCAGAC -3' and reverse, 5'- CGAGCTTTAAAAGATAGTTCC -3') described by Awad and colleagues.[3] After the process of sequencing, the sequences of each allele were perceived using CHROMAS program.

Statistical analysis

Allele and genotype frequencies were determined by direct counting and then divided by the number of chromosomes to produce an allele frequency, or by the number of subjects to produce the genotype frequency. The goodness of fit to Hardy-Weinberg equilibrium, calculating the expected frequencies of each genotype and comparing them with the observed values, was performed using a chi-square test. Allele and genotype frequencies were compared between populations using Chi-square (χ^2) test. A p value of <0.05 was considered significant. Chi-square and p value were calculated using the statistical program Epi Info version 6 (http://www.CDC.gov/epiinfo/Epi6/ei6.htm).

Result

Polymorphism at +874A/T in the first intron of the IFN-γ gene was identified using PCR-SSP method. Representative results of a PCR-SSP pattern and chromatogram of DNA sequences are shown in Figure 1 and 2, respectively. Genotype and allele frequencies for the + 874 at the first intron of IFN-γ gene are shown in table 1. Eighty- three of 137 healthy Thai individuals (60.6 %) were homozygous for A/A genotype; 46 (33.6 %) were heterozygous; and, 8 (5.8 %) were homozygous for T/T genotype. Genotype frequencies of healthy Thai individuals were in Hardy-Weinberg equilibrium. The distributions of the cytokine gene polymorphisms between a Thai population and other populations in a previous report are compared (Table 2). There were significant differences in allele and genotype frequencies between Thai population with Italian ($\chi^2 = 9.8$; $p = 0.001$ and $\chi^2 = 20.9$; $p = 0.00002$, respectively), and Brazilian Caucasian ($\chi^2 = 4.6$; $p = 0.03$ and $\chi^2 = 12$; $p = 0.002$, respectively). In contrast, there were no significant differences in allele and genotype frequencies between Thai population with South African and Asian.[12, 15]

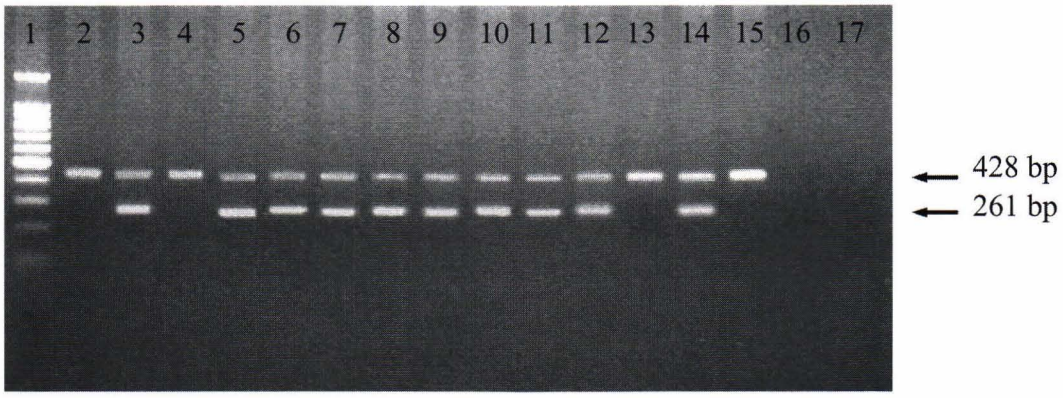

Figure 1. The representative result from samples with homozygous of +874T, heterozygous +874T/A and homozygous of +874A.
Lane 1 is 100 bp molecular marker.
Lane 2-5 (2-3 sample N7 and 4-5 sample N14) are homozygous of +874T.
Lane 6-11 (6-7sample N9, 8-9 sample N11 and 10-11 sample N17) are heterozygous +874T/A.
Lane 12-15 (12-13 sample N5 and 4-15 sample N6) are homozygous of +874A.
Lane 16-17 are Negative control of in each specific primer (NA is Negative control of +874A allele specific primer and NT is Negative control of +874T allele specific primer).

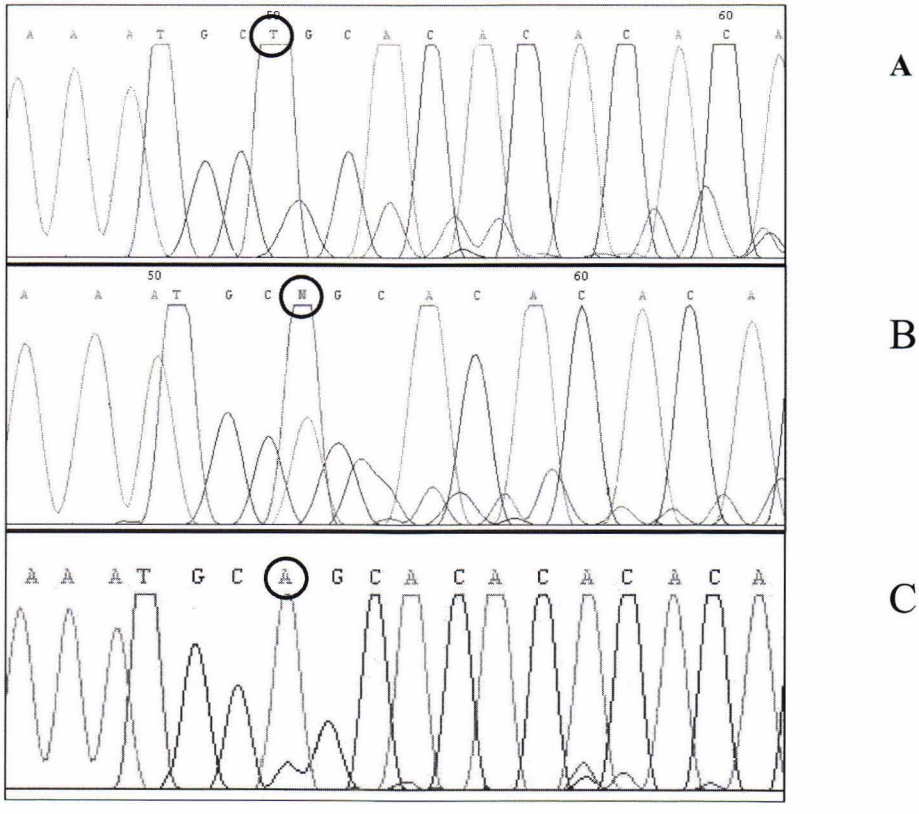

Figure 2. Chromatogram of DNA sequences in homozygous of +874T, heterozygous +874T/A and homozygous of +874A. Homozygous of +874T from sample N7 (A), heterozygous +874T/A from sample N9 (B) and homozygous of +874A from sample N5 (C).

Table 1. Genotype and allele frequencies for the +874T/A at the first intron of IFN-γ gene in healthy Thai individuals.

IFN-γ (+874T/A)	Healthy Thai individuals (n = 137)
Genotype frequencies	
A/A	83 (60.6 %)
A/T	46 (33.6 %)
T/T	8 (5.8 %)
Allele frequencies	
A	212 (77.4 %)
T	62 (22.6 %)

Table 2. Comparison between genotype and allele frequencies of IFN-γ gene polymorphism in the different population.

Cytokine (Position)		Control				
	Author	Poli et al.[13]	Daher et al.[14]	Govan et al.[15]	Hoffmann et al.[12]	Present study
	Year	2002	2003	2003	2002	2006
	Ethnic group	Italian [a, c]	Brazilian Caucasian [b, d]	South African	Asian	Thai
	N	363	104	140	29	137
	Genotype					
FN-γ (+874)	A/A	116 (32 %)	39 (38 %)	102 (73 %)	19 (66 %)	83 (60.6 %)
	A/T	170 (46.8 %)	50 (48 %)	31 (22 %)	7 (24 %)	46 (33.6 %)
	T/T	77 (21.2 %)	15 (14 %)	7 (5 %)	3 (10 %)	8 (5.8 %)
	Allele					
	A	402 (55.3 %)	128 (61.5 %)	235 (84 %)	45 (77.6 %)	212 (77.4 %)
	T	324 (44.7 %)	80 (38.5 %)	45 (16 %)	13 (22.4 %)	62 (22.6 %)

[a] compare between allele frequencies in Thai with Italian ; χ^2 =9.8 , p=0.001

[b] compare between allele frequencies in Thai with Brazilian Caucasian ; χ^2 =4.6 , p=0.03

[c] compare between genotype frequencies in Thai with Italian ; χ^2 =20.9 , p=0.00002

[d] compare between genotype frequencies in Thai with Brazilian Caucasian ; χ^2 =12 , p=0.002

There were no significant differences in allele and genotype frequencies between Thai population with South African and Asian

Discussion

This study has genotyped unrelated healthy Thai individuals for +874A/T in the first intron of the IFN-γ gene and compared the results with those published for other populations. The distribution of polymorphisms examined in this study was similar to that observed in Asian and South African. In contrast, there are significant difference between Thais with Italians and Brazilian Caucasians. In Asian population, the distribution of +874T allele, which is associated with high IFN-γ production, is lower than in Caucasians (22 % in Asians and 38 - 44 % in Caucasians). Our group and other reported the difference in the distributions of some cytokine gene polymorphisms between Asians and other populations.[10-12]

IFN-γ gene at +874 polymorphisms has been reported in association with the susceptibility and clinical severity of disease in several studies such as tuberculosis, breast cancer, chronic allograft nephropathy and *Helicobacter pylori* disease outcome.[5-8] However, the results varied in each ethnic group. For example, the results from case-control study in tuberculosis which the +874T/T genotype was significant decrease in the Sicilian patients[16], but not associated with patients from Croatian Caucasians.[5] Therefore, the ethnic difference in the distribution pattern of IFN-γ polymorphism may explain the ethnic differences in susceptibility or severity of many diseases. However, only SNP at one position in the gene might not absolutely control gene expression. Therefore, study of haplotype analysis (combination of SNPs in the same chromosome) is necessary. A more complete knowledge of specific polymorphisms influence levels of cytokine expression and production could provide clinicians with ability to optimize regimens based upon a cytokine profile of individuals.

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