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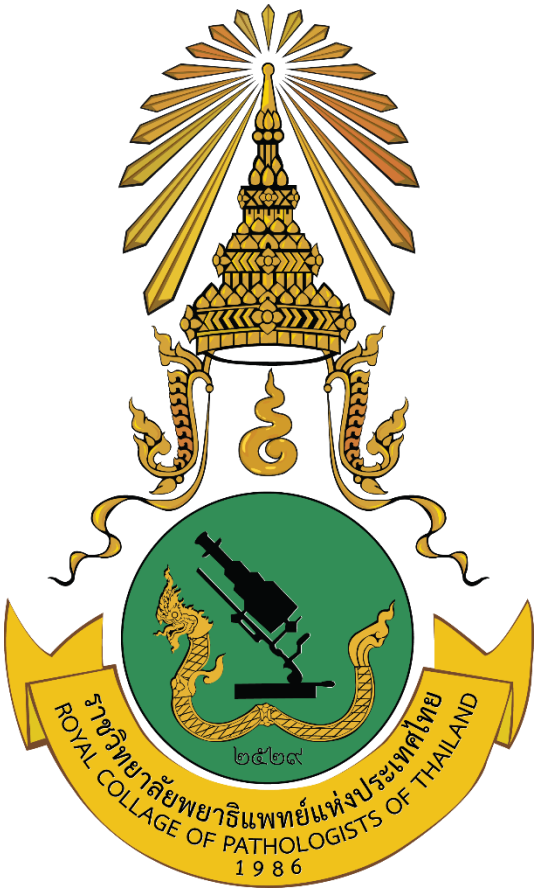
THE OFFICIAL JOURNAL OF THE ROYAL COLLEGE OF PATHOLOGISTS OF THAILAND



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ABOUT THE JOURNAL

Aims and Scope

Asian Archives of Pathology (AAP) is an open access, peer-reviewed journal. The journal was first published in 2002 under the Thai name "วารสารราชวิทยาลัยพยาธิแพทย์แห่งประเทศไทย" and English name "Journal of the Royal College of Pathologists of Thailand". The journal is a publication for workers in all disciplines of pathology and forensic medicine. In the first 3 years (volumes), the journal was published every 4 months. Until 2005, the journal has changed its name to be "Asian Archives of Pathology: The Official Journal of the Royal College of Pathologists of Thailand", published quarterly to expand the collaboration among people in the fields of pathology and forensic medicine in the Asia-Pacific regions and the Western countries.

The full articles of the journal are appeared in either Thai or English. However, the abstracts of all Thai articles are published in both Thai and English languages. The journal features letters to the editor, original articles, review articles, case reports, case illustrations, and technical notes. Diagnostic and research areas covered consist of (1) **Anatomical Pathology** (including cellular pathology, cytopathology, haematopathology, histopathology, immunopathology, and surgical pathology); (2) **Clinical Pathology (Laboratory Medicine)** [including blood banking and transfusion medicine, clinical chemistry (chemical pathology or clinical biochemistry), clinical immunology, clinical microbiology, clinical toxicology, cytogenetics, parasitology, and point-of-care testing]; (3) **Forensic Medicine (Legal Medicine or Medical Jurisprudence)** (including forensic science and forensic pathology); (4) **Molecular Medicine** (including molecular genetics, molecular oncology, and molecular pathology); (5) **Pathobiology**; and (6) **Pathophysiology**.

All issues of our journal have been printed in hard copy since the beginning. Around the late 2014, we developed our website (www.asianarchpath.com) in order to increase our visibility. We would like to acknowledge that our journal has been sponsored by the Royal College of Pathologists of Thailand. We have the policy to disseminate the verified scientific knowledge to the public on a non-profit basis. Hence, we have not charged the authors whose manuscripts have been submitted or accepted for publication in our journal.

On the other hand, if any authors request a printed copy of the journal issue containing the articles, each of the copied journals costs 450 bahts for Thai authors and 30 United States dollars (USD) for international authors.

Publication Frequency

Four issues per year

Disclaimer

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LETTER TO THE EDITOR

ผู้ช่วยพยาธิแพทย์ (Pathologists' assistants)

ธีรยสถ์ นิมมานนท์

ภาควิชาพยาธิวิทยา ชั้น 6 อาคารเจ้าฟ้าเพชรรัตน วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า
เลขที่ 317 ถนนราชวิถี แขวงทุ่งพญาไท เขตราชเทวี จังหวัดกรุงเทพมหานคร รหัสไปรษณีย์ 10400
โทรศัพท์: +66 (0) 89 050 7776 และ +66 (0) 95 175 3052 โทรสาร: +66 (0) 2 354 7791
Email: thirayost@pcm.ac.th, thirayost@pcmpathology.org, thirayost@outlook.com

ถึงแม้ว่า ณ ปัจจุบันนี้จะยังไม่มีข้อมูลหลักฐานอันเป็นที่ประจักษ์ แต่ก็เป็นที่ทราบกันโดยทั่วไปว่าพยาธิแพทย์ในประเทศไทยซึ่งปฏิบัติงานอยู่ในหน่วยงานของภาครัฐและเอกชนต่างประสบปัญหาภาระงานที่มากเกินพอดีในการตรวจสิ่งส่งตรวจทางพยาธิวิทยากายวิภาคด้วยตาเปล่าและกล้องจุลทรรศน์ โดยเฉพาะพยาธิแพทย์ผู้ทำหน้าที่เป็นอาจารย์ในโรงเรียนแพทย์ต้องมีภาระงานด้านการเรียนการสอนแก่นักเรียนแพทย์และแพทย์ประจำบ้านสาขาพยาธิวิทยากายวิภาค (Anatomical Pathology) และ/หรือสาขาพยาธิวิทยาทั่วไป (Anatomical and Clinical Pathology) ร่วมกับปฏิบัติงานด้านการวิจัยเพิ่มเติมด้วย ดังนั้นจึงปรากฏความต้องการจากพยาธิแพทย์เป็นจำนวนมากสำหรับบุคคลซึ่งปฏิบัติหน้าที่เป็น "ผู้ช่วยพยาธิแพทย์ (Pathologists' assistant)" เพื่อแบ่งเบาภาระงานด้านต่าง ๆ ของพยาธิแพทย์[1-3] อันประกอบไปด้วย

1. การติดต่อสื่อสารและประสานงานกับหน่วยงานต่าง ๆ ทั้งภายในและภายนอกสถาบัน สำหรับขั้นตอนการเก็บสิ่งส่งตรวจทางพยาธิวิทยากายวิภาค การจัดส่ง และการรายงานผล
2. การตรวจสอบความครบถ้วนและความถูกต้องของสิ่งส่งตรวจทางพยาธิวิทยากายวิภาคก่อนรับเข้ามาในระบบ รวมถึงการเก็บรักษาตามระยะเวลาที่กำหนดของสิ่งส่งตรวจภายหลังการตรวจด้วยตาเปล่าเป็นที่เรียบร้อยแล้ว
3. การบรรยายพร้อมกับการถ่ายภาพของพยาธิสภาพและ/หรือรอยโรคของสิ่งส่งตรวจที่พบขณะทำการตรวจทางพยาธิวิทยากายวิภาคด้วยตาเปล่า
4. การตรวจสอบ จัดเตรียม ดูแลรักษา และทำความสะอาดอุปกรณ์ที่เกี่ยวข้องกับการตรวจสิ่งส่งตรวจทางพยาธิวิทยากายวิภาคด้วยตาเปล่าให้อยู่ในสภาพสมบูรณ์และพร้อมใช้อยู่เสมอ

5. การเก็บ การลงทะเบียน การคัดแยก การดูแลรักษา รวมถึงการบริหารจัดการสิ่งส่งตรวจประเภทต่าง ๆ สำหรับการนำไปใช้ในงานวิจัยทางด้านชีวเวชศาสตร์ (Biomedical sciences) พยาธิวิทยา (Pathology) พยาธิชีววิทยา (Pathobiology) อณูเวชศาสตร์ (Molecular Medicine) และอณูพยาธิวิทยา (Molecular pathology)

อนึ่งการปฏิบัติงานของผู้ช่วยพยาธิแพทย์ในการตรวจสิ่งส่งตรวจทางพยาธิวิทยากายวิภาคด้วยตาเปล่ามีประสิทธิผลเทียบเคียงหรือมากกว่าแพทย์ประจำบ้านสาขาพยาธิวิทยากายวิภาคและ/หรือสาขาพยาธิวิทยาทั่วไป[4] เพราะฉะนั้นผู้ช่วยพยาธิแพทย์ที่มีความชำนาญการนั้นสามารถที่จะมีบทบาทและร่วมเป็นส่วนหนึ่งในการช่วยเหลือและการให้คำแนะนำเรื่องการตรวจสิ่งส่งตรวจทางพยาธิวิทยากายวิภาคด้วยตาเปล่าสำหรับแพทย์ประจำบ้านที่เพิ่งเข้ารับการฝึกอบรมซึ่งจะยังผลให้เกิดประสิทธิภาพของกระบวนการเรียนรู้ที่เพิ่มขึ้นของแพทย์ประจำบ้านอันเนื่องมาจากการแลกเปลี่ยนความคิดเห็นและการอภิปรายถกเถียงทางวิชาการซึ่งกันและกัน อีกทั้งยังก่อให้เกิดการลดภาระงานของพยาธิแพทย์ผู้เป็นอาจารย์ได้ส่วนหนึ่งด้วย[1-3,5]

ด้วยเหตุผลดังกล่าวมาข้างต้นทั้งหมดการฝึกอบรมบุคคลเพื่อมาปฏิบัติหน้าที่เป็นผู้ช่วยพยาธิแพทย์จึงเป็นสิ่งที่จำเป็นอย่างยิ่งยวดและเร่งด่วนสำหรับสถานการณ์ปัจจุบันทางแพทยศาสตร์และสาธารณสุขศาสตร์ของประเทศไทย

<u>เอกสารอ้างอิง</u>

(1). Grzybicki, D.M., Reilly, T.L., Hart, A.R., Galvis, C.O. & Raab, S.S. 2001, "National practice characteristics and utilization of pathologists' assistants", *Archives of Pathology & Laboratory Medicine,* vol. 125, no. 7, pp. 905-912.

(2). Grzybicki, D.M., Vrbin, C.M., Reilly, T.L., Zarbo, R.J. & Raab, S.S. 2004, "Use of physician extenders in surgical pathology practice", *Archives of Pathology & Laboratory Medicine,* vol. 128, no. 2, pp. 165-172.

(3). Vitale, J., Brooks, R., Sovocool, M. & Rader, W.R. 2012, "Value-added benefits and utilization of pathologists' assistants", *Archives of Pathology & Laboratory Medicine,* vol. 136, no. 12, pp. 1565-1570.

(4). Galvis, C.O., Raab, S.S., D'Amico, F. & Grzybicki, D.M. 2001, "Pathologists' assistants practice: a measurement of performance", *American Journal of Clinical Pathology,* vol. 116, no. 6, pp. 816-822.

(5). Grzybicki, D.M. & Vrbin, C.M. 2003, "Pathology resident attitudes and opinions about pathologists' assistants", *Archives of Pathology & Laboratory Medicine,* vol. 127, no. 6, pp. 666-672.

ORIGINAL ARTICLE

The X-chromosomal short tandem repeats analysis in Thai population

Wittawat Chantkran[1] and Budsaba Rerkamnuaychoke[2*]

[1] *Department of Pathology, Phramongkutklao College of Medicine, Bangkok, Thailand*

[2] *Human Genetics Unit, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

* Correspondence to: Associate Professor Dr Budsaba Rerkamnuaychoke, Human Genetics Unit, Department of Pathology, Floor 4, Main Building, Faculty of Medicine Ramathibodi Hospital, Mahidol University, 270 Rama VI Road, Rajadevi, Bangkok, 10400 Thailand. Telephone: +66 (0) 2 201 1368 and +66 (0) 2 201 1369 Fax: +66 (0) 2 201 1464 Email: budsaba.rer @mahidol.ac.th, rabrk@mahidol.ac.th

Conflict of interest: The authors declare that they have no conflicts of interest with the contents of this article.

Abstract

Human variations in the X chromosome are useful tools in studying human genetic diversity and individual identification. Five X chromosomal short tandem repeats (X-STRs) multiplex system (DXS8378, DXS101, HPRTB, DXS8377 and DXS10011) were amplified in one single polymerase chain reaction. DNA samples of 200 (101 males and 99 females) unrelated healthy individuals Thai, and 15 family trios with female children, were successfully analysed using this five X-STRs multiplex system. The distributions of allele frequencies were examined for independence. When the forensic efficiency was calculated, DXS10011 locus was found to be the greatest marker for forensic application and population study. The combined powers of discrimination of five loci in males and females were 0.999993 and 0.999999, respectively. These five X chromosome markers are highly informative for population study and the database of Thailand.

Keywords: X chromosome; short tandem repeats; multiplex PCR; Thai population; forensics

Introduction

For many years, autosomal and Y-chromosomal DNA have been used for forensic purposes[1,2]. At the present time, X-chromosomal markers have been observed as a forensic of interest[3-7]. The availability of the finished sequence of the human X chromosome[8], now allows exploring its evolution and unique properties at a new level. The X chromosome contains about 5% of the haploid genome and is completely conserved in gene content between species. However, evolutionary processes are likely to have shaped the behaviour and structure of the X chromosome in many other ways, influencing features such as repeat content, gene content, mutation rate and haplotype structure.

Study results of variations in the X chromosome are invaluable tools for studying the human genetic diversity and individual identification. The X-STRs may strengthen the results of autosomal and Y-STRs analysis. In paternity testing, although the X-STRs are advantageous only in case the alleged child is female, the X-STRs often have a greater power of exclusion than autosomal markers. Besides, the X-STRs are very beneficial in maternity testing or in deficiency paternity cases, for instance, to judge the paternity of disputed half-sisters with the same father in case the mothers' DNA are unavailable[5].

The X-chromosome is inherited via a sex-based pattern. Because the recombination of the X chromosome occurs only in females, there is a less effective population size, greater linkage disequilibrium, and a stronger genetic drift. These factors bring the X chromosome to be a source of data in human evolution and evacuation studies[8].

In this study, five X chromosome markers of interest in forensic science will be typed in the Thai population in order to report population database and haplotype profiling in the Thai population and calculating the population genetic parameters for these markers.

Materials and Methods

DNA extraction:

DNA extraction from whole blood samples of 200 Thai people (101 males and 99 females) were performed with a commercial method, according to manufacturer's instructions (Promega Corp., Madison, WI, USA). An aliquot containing approximately 4 ng of DNA was used in each PCR amplification. In addition, 15 family trios with female children (previously confirmed by autosomal STRs analysis) were checked for regular X-chromosomal inheritance.

STR amplification and fragment analysis:

After various tests with different STRs and optimisation of PCR parameters, the STRs DXS8378, DXS101, HPRTB, DXS8377 and DXS10011 were combined into the PCR multiplex system. Primer sequences, labeling, concentrations, and references are shown in *Table 1*. Besides the primers, the PCR reaction mix (12.5 µl reaction volume) contained 1X AmpliTaq buffer II, 1.5 mM of $MgCl_2$, 200 µl of each dNTP, and 2 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster city, CA, USA). A GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster city, CA, USA) was used. After several pilot experiments to optimise the amplification of all markers in the same tube, a PCR touch-down protocol was chosen[9], consisting of an initial denaturation step at 95 °C for 10 min, followed by nine cycles with denaturation at 94 °C for 30 s, annealing at decreasing temperature between 61 and 65 °C (decreasing 0.5 °C each cycle) for 1 min 30 s and extension at 72 °C for 1 min 15 s. Then 28 cycles at 94 °C 30 s, 58 °C 1 min 30 s, 72 °C 1 min 15 s; followed by a final extension at 60 °C for 60 min.

Table 1 Primer sequences, concentrations, dye labeling and references.

Locus	Primer sequence (5' → 3')	Label	Concentration (µM)	Reference
DXS8378	CACAGGAGGTTTGACCTGTT AACTGAGATGGTGCCACTGA	PET	0.2	GDB, (15)
DXS101	ACTCTAAATCAGTCCAAATATCT AAATCACTCCATGGCACATGTAT	NED	2.5	GDB, (4)
HPRTB	TCTCTATTTCCATCTCTGTCTCC TCACCCCTGTCTATGGTCTCG	6-FAM	0.3	GDB, (15)
DXS8377	ACCACTTCATGGCTTACCACAG TATGGACCTTTGGAAAGCTAG	VIC	0.08	GDB, (16)
DXS10011	CTGAGATTGCACCATTGCAC TGGGAGAACCGTTTGAAGTT	6-FAM	0.5	GDB, (13)

Note: *GDB = Genome database (www.gdb.org)*

An aliquot containing 1 µl of PCR product was mixed with 10 µl of Hi-Di formamide and 0.5 µl of GeneScan 500LIZ size standard (Applied Biosystems), heated at 95 °C for 3 min, quenched at 4 °C for 3 min and injected into an Applied Biosystems 3130 Genetic Analyzer.

Fragment sizes were automatically determined using GeneScan analysis software (Applied Biosystems). Genotyping was analysed using GeneMapper ID software (Applied Biosystems) by comparison with reference DNA control sample 9947A (female) (Applied Biosystems). Alleles were assigned according to the recommendations of the International Society of Forensic Haemogenetics (ISFH) commission[10].

Statistical analysis:

Allelic frequencies were calculated by using the data from males and females collectively and observed heterozygosity (HETobs) were calculated using the female data by Power Stat V12 program (www.promega.com). Mean exclusion chance (MEC), power of discrimination for females (PDf) and power of discrimination for males (PDm) were computed by chromosome X web software (www.chrx-str.org). The combined power of discrimination was determined as 1-[(1-PDa)•(1-PDb)•(1-PDc)•(1-PDd)•••] with PDa-d indicating the discriminating power of the different polymorphism[11]. To evaluate Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD), GENEPOP program (version 3.4) was used.

Results

The 9947A DNA (female) was used as a control reference sample. The allele frequencies for the X-chromosomal STRs DXS8378, DXS101, and HPRTB are displayed in *Table 2*, the data for DXS8377and DXS10011 in *Table 3*. The forensic efficiency of the five X-STRs loci were calculated (*Table 4*). DXS8378 had the lowest values for PDm, PDf, and MEC in the present study. The combined power of discrimination in males and females were 0.999993 and 0.999999, respectively. Observed heterozygosity (HETobs) in females for the five markers ranged from 0.660 to 0.900 in this study, DXS8377 had the highest whereas DXS8378 had the lowest HETobs.

An exact test for Hardy-Weinberg equilibrium (HWE) performed on female samples indicated that the genotype distributions did not deviate from HWE at any of the loci, except for threshold significance at locus DXS10011 (P=0.0000; data not shown). When exact tests showed no significant difference between allele distributions of the two groups, pooled male and female databases of the Thai populations were purposely created to compare the allele frequencies at locus DXS8378, DXS101, HPRTB, DXS8377, and DXS10011 between different races (*Tables 5 – 9*).

Table 2 Allele frequencies for the X-chromosomal STRs DXS8378, DXS101 and HPRTB in Thai population. Allele frequencies were collected from the analysis of DNA samples of 200 (101 males and 99 females) unrelated healthy individuals Thai.

Allele	DXS8378		DXS101		HPRTB	
	Male	Female	Male	Female	Male	Female
8		0.005				
9	0.020	0.020				
10	0.475	0.525				
11	0.337	0.247			0.208	0.136
12	0.149	0.177			0.238	0.263
13	0.020	0.025			0.317	0.348
14					0.218	0.217
15					0.020	0.035
16						
17						
18			0.010			
19			0.020			
21			0.010			
22			0.069	0.066		
23			0.079	0.051		
24			0.208	0.247		
25			0.257	0.283		
26			0.178	0.192		
27			0.069	0.086		
28			0.050	0.061		
29			0.040	0.010		
30			0.010	0.005		

Table 3 Allele frequencies for the X-chromosomal STRs DXS8377 and DXS10011 in Thai population. Allele frequencies were collected from the analysis of DNA samples of 200 (101 males and 99 females) unrelated healthy individuals Thai.

Allele	DXS8377		DXS100011	
	Male	Female	Male	Female
27				0.015
28			0.020	0.005
29.2			0.020	0.025
30			0.069	0.056
30.2			0.030	0.015
31				0.035
31.2				0.015
32			0.030	0.025
32.2			0.020	0.010
33			0.050	0.030
33.2			0.020	0.045
34			0.089	0.040
34.2			0.010	0.010
35			0.030	0.071
35.2			0.040	0.010
36			0.050	0.081
37			0.069	0.056
37.2			0.010	
38			0.109	0.086

Table 3 (Continued) Allele frequencies for the X-chromosomal STRs DXS8377 and DXS10011 in Thai population. Allele frequencies were collected from the analysis of DNA samples of 200 (101 males and 99 females) unrelated healthy individuals Thai.

Allele (Continued)	DXS8377		DXS100011	
	Male	Female	Male	Female
39			0.050	0.056
40			0.050	0.061
41	0.010		0.050	0.040
42	0.010	0.010	0.050	0.076
43	0.020	0.010	0.040	0.020
44	0.030	0.045	0.030	0.035
45	0.059	0.061	0.010	0.040
46	0.099	0.111	0.040	0.005
47	0.139	0.162		0.010
48	0.089	0.131	0.010	
49	0.129	0.121		0.020
50	0.099	0.101		0.005
51	0.129	0.071	0.010	
52	0.059	0.035		
53	0.050	0.061		
54	0.059	0.030		
55	0.010	0.035		
56	0.010	0.010		
57		0.005		

Table 4 Statistical parameters of forensic interest for X-chromosomal STRs in Thai population. Statistical parameters were analysed from 200 (101 males and 99 females) unrelated healthy individuals Thai shown in *Table 2* and *3*. HETobs, HETexp and PDf were analysed from females only. PDm was analysed from males only.

Allele	DXS8378	DXS101	HPRTB	DXS8377	DXS10011
PIC	0.570	0.780	0.700	0.890	0.950
HETobs	0.660	0.710	0.680	0.900	0.670
HETexp	0.627	0.799	0.738	0.896	0.944
MEC I	0.375	0.619	0.505	0.799	0.894
MEC II	0.574	0.777	0.698	0.892	0.944
MEC III	0.575	0.777	0.699	0.893	0.946
MEC IV	0.427	0.654	0.559	0.814	0.901
PDm	0.638	0.838	0.752	0.905	0.946
PDf	0.800	0.932	0.888	0.970	0.979

Note: *HETexp = Expected heterozygosity; HETobs = Observed heterozygosity; MEC = Mean exclusion chance (I for autosomal markers; II for X-chromosomal markers in trios; III for X-chromosomal markers in trios; and IV for X-chromosomal markers in father/daughter pairs); PDf = Power of discrimination in females; PDm = Power of discrimination in males; and PIC = Polymorphism information content*

Table 5 Allele frequencies, statistical parameters and references of DXS8378 in six populations. For the Thai population, the data were from pooled males and females (*Table 2*) when the exact tests showed no difference between the two groups. Allele 10 was observed as a most common allele shared among the Asian population (Thai, Japan and China) and allele 8 was found only in Thai population. Some statistical parameters were not available.

Parameter		Thai	Japan	China	Italy	Angola	Mozambique
Sex	Male	101	195	250	100	74	112
	Female	99	138	250	100	0	0
Allele	8	0.003					
	9	0.020	0.015	0.028	0.020		0.009
	10	0.500	0.562	0.521	0.342	0.189	0.295
	11	0.293	0.285	0.275	0.520	0.378	0.348
	12	0.163	0.113	0.149	0.242	0.324	0.321
	13	0.023	0.025	0.023	0.040	0.108	0.027
	14			0.004	0.003		
	PDm	0.638	0.574	0.633	0.686	0.714	0.694
	PDf	0.800	0.776	0.803	0.865	0.858	0.838
	MEC II/III	0.575	—	—	0.646	0.650	0.624
	MEC IV	0.427	—	—	—	0.505	0.477
Reference		—	(18)	(20)	(22)	(25)	(25)

Note: *MEC = Mean exclusion chance (II for X-chromosomal markers in trios; III for X-chromosomal markers in trios; and IV for X-chromosomal markers in father/daughter pairs); PDf = Power of discrimination in females; and PDm = Power of discrimination in males*

Table 6 Allele frequencies, statistical parameters and references of DXS101 in six populations. For the Thai population, the data were from pooled males and females (*Table 2*) when the exact tests showed no difference between the two groups. No significant most common allele shared in any population was observed. Some statistical parameters were not available.

Parameter		Thai	Japan	German	Italy	Angola	Mozambique
Sex	Male	101	195	216	70	74	112
	Female	99	138	348	70	0	0
Allele	14			0.002			0.009
	15			0.044	0.029		0.009
	16			0.005	0.010		
	17			0.002	0.005		
	18	0.005		0.084	0.095	0.027	0.027
	19	0.010		0.047	0.033	0.081	0.045
	20				0.043	0.081	0.080
	21	0.005	0.002	0.012	0.057	0.108	0.232
	22	0.068	0.040	0.032	0.024	0.068	0.063
	23	0.065	0.119	0.022	0.048	0.081	0.089
	24	0.228	0.304	0.066	0.171	0.068	0.134
	25	0.270	0.217	0.212	0.167	0.068	0.054
	26	0.185	0.176	0.156	0.152	0.162	0.071
	27	0.078	0.091	0.114	0.076	0.135	0.116
	28	0.055	0.036	0.079	0.067	0.095	0.018
	29	0.025	0.013	0.070	0.024	0.014	0.036
	29.2					0.014	0.018
	30	0.008	0.002	0.027			
	31			0.024			
	32			0.001			

Table 6 (Continued) Allele frequencies, statistical parameters and references of DXS101 in six populations. For the Thai population, the data were from pooled males and females (*Table 2*) when the exact tests showed no difference between the two groups. No significant most common allele shared in any population was observed. Some statistical parameters were not available.

Parameter		Thai	Japan	German	Italy	Angola	Mozambique
Allele (Continued)	PDm	0.838	0.798	0.889	—	0.913	0.892
	PDf	0.932	0.937	0.978	—	0.982	0.977
	MEC II/III	0.777	—	0.879	—	0.892	0.874
	MEC IV	0.654	—	—	—	0.812	0.786
Reference		—	(18)	(4)	(23)	(25)	(25)

Note: *MEC = Mean exclusion chance (II for X-chromosomal markers in trios; III for X-chromosomal markers in trios; and IV for X-chromosomal markers in father/daughter pairs); PDf = Power of discrimination in females; and PDm = Power of discrimination in males*

Six alleles were identified at locus DXS8378, ranging from allele 8 to 13, the most common being 10 (*Table 2*). Allele 10 was also observed as a most common allele shared among the Asian population (Thai, Japan and China) and allele 8 was found only in the Thai population (*Table 5*). Twelve alleles were found at locus DXS101, ranging from allele 18 to 30, the most common being 25 (*Table 2*). The comparison of allele frequencies at DXS101 locus between different races showed no significant most common allele shared in any population (*Table 6*). Five alleles ranging from allele 11 to 15 were identified at locus HPRTB, the most common allele being 13 (*Table 2*). Allele 13 was observed as a most common allele shared among the Asian population (Thai, Japan and China) (*Table 7*). DXS8377 had 17 alleles ranging from 41 to 57. The most common allele was 47 (*Table 3*). Among the African population (Angola, Uganda and Mozambique), a shared most common allele was 49 (*Table 8*). DXS10011 had 32 alleles ranging from 27 to 51, the most common allele was 38 (*Table 3*). There was no significant most common allele shared between different races at locus DXS10011 (*Table 9*).

Table 7 Allele frequencies, statistical parameters and references of HPRTB in six populations. For the Thai population, the data were from pooled males and females (*Table 2*) when the exact tests showed no difference between the two groups. Allele 13 was observed as a most common allele shared among the Asian population (Thai, Japan and China). Some statistical parameters were not available.

Parameter		Thai	Japan	China	Latvia	Angola	Mozambique
Sex	Male	101	229	250	78	74	112
	Female	99	172	250	45	0	0
Allele	9				0.007		
	10				0.014		
	11	0.173	0.033	0.092	0.149		0.027
	12	0.250	0.269	0.292	0.365	0.068	0.098
	13	0.333	0.490	0.391	0.297	0.216	0.348
	14	0.218	0.140	0.173	0.135	0.351	0.277
	15	0.028	0.056	0.047	0.014	0.243	0.188
	16		0.012	0.004	0.007	0.108	0.063
	17			0.001	0.014	0.014	
	PDm	0.752	0.694	0.705	0.737	0.765	0.760
	PDf	0.888	0.819	0.866	0.889	0.900	0.899
	MEC II/III	0.699	0.614	—	0.778	0.715	0.713
	MEC IV	0.559	—	—	0.656	0.578	0.576
Reference		—	(17)	(20)	(24)	(25)	(25)

Note: *MEC = Mean exclusion chance (II for X-chromosomal markers in trios; III for X-chromosomal markers in trios; and IV for X-chromosomal markers in father/daughter pairs); PDf = Power of discrimination in females; and PDm = Power of discrimination in males*

Table 8 Allele frequencies, statistical parameters, and references of DXS8377 in six populations. For the Thai population, the data were from pooled males and females (*Table 3*) when the exact tests showed no difference between the two groups. Allele 49 was observed as a most common allele shared among the African population (Angola, Uganda and Mozambique). Some statistical parameters were not available.

Parameter		Thai	Japan	Latvia	Angola	Uganda	Mozambique
Sex	Male	101	229	78	74	51	112
	Female	99	172	45	0	0	0
Allele	36					0.020	0.009
	37				0.014	0.020	
	40			0.007		0.020	0.018
	41	0.005		0.007	0.014	0.020	0.018
	42	0.010	0.012	0.027	0.054	0.020	0.071
	43	0.015	0.028	0.047	0.054	0.118	0.071
	44	0.038	0.024	0.020	0.068	0.039	0.080
	45	0.060	0.070	0.068	0.095	0.137	0.080
	46	0.105	0.100	0.074	0.041	0.078	0.106
	47	0.150	0.129	0.068	0.068	0.078	0.045
	48	0.110	0.147	0.169	0.095	0.098	0.036
	49	0.125	0.171	0.128	0.108	0.177	0.107
	50	0.100	0.105	0.115	0.068	0.020	0.089
	51	0.100	0.063	0.101	0.041	0.020	0.098
	52	0.048	0.047	0.061	0.095	0.098	0.018
	53	0.055	0.030	0.061	0.027		0.071
	54	0.045	0.033	0.014	0.068		0.018
	55	0.023	0.026	0.027	0.081	0.020	0.045
	56	0.010	0.005	0.007	0.014	0.020	0.009
	57	0.003	0.007				
	58		0.003				0.009

Table 8 (Continued) Allele frequencies, statistical parameters, and references of DXS8377 in six populations. For the Thai population, the data were from pooled males and females (*Table 3*) when the exact tests showed no difference between the two groups. Allele 49 was observed as a most common allele shared among the African population (Angola, Uganda and Mozambique). Some statistical parameters were not available.

Parameter		Thai	Japan	Latvia	Angola	Uganda	Mozambique
Allele (Continued)	PDm	0.905	0.887	0.905	0.939	0.918	0.933
	PDf	0.970	0.982	0.983	0.990	0.982	0.989
	MEC II/III	0.893	0.887	0.897	0.921	0.891	0.920
	MEC IV	0.814	—	0.821	0.856	0.821	0.856
Reference		—	(17)	(24)	(25)	(25)	(25)

Note: *MEC = Mean exclusion chance (II for X-chromosomal markers in trios; III for X-chromosomal markers in trios; and IV for X-chromosomal markers in father/daughter pairs); PDf = Power of discrimination in females; and PDm = Power of discrimination in males*

Discussions

Individuals from the Thai population including 101 males, 99 females and 15 family trios with female children were successfully investigated with five X chromosome markers. The multiplex PCR assays were optimized for a DNA concentration of 4 ng initially but later were able to be diluted to 2 ng, that is, they were sensitive enough for routine paternity analysis. The DXS10011 locus was highly polymorphic, with the highest power of discrimination and probability of paternity exclusion among the five markers studied.

When the comparison of allele frequencies between different races was observed, the Asian population shared the most common allele, that is, allele 10 at locus DXS8378 and allele 13 at locus HPRTB whereas allele 49 at locus DXS8377 was a most common allele shared among the African population. DXS10011 had the greatest individual value as a forensic marker, with the highest power of discrimination in males (PDm) and females (PDf), as well as mean exclusion chance.

Table 9 Allele frequencies, statistical parameters, and references of DXS10011 in six populations. For the Thai population, the data were from pooled males and females (*Table 3*) when the exact tests showed no difference between the two groups. No significant most common allele shared in any population was observed. Some statistical parameters were not available.

Parameter		Thai	Japan	Taiwan	Latvia	German	Algeria
Sex	Male	101	56	92	78	105	104
	Female	99	48	181	45	200	106
Allele	17		0.007				
	18		0.007	0.005			
	19		0.020	0.025			
	20		0.026	0.047			
	21		0.020	0.047			0.003
	21.2		0.013	0.008			
	22		0.066	0.049			
	22.2		0.046	0.011			
	23		0.026	0.030			
	23.2		0.039	0.047			
	24		0.046	0.041			
	24.2		0.072	0.049			
	25		0.020	0.049			
	25.2		0.026	0.038			
	26		0.046	0.027			0.003
	26.2		0.020	0.005			
	27	0.008	0.020	0.033			0.013
	27.2		0.013	0.008			
	28	0.013	0.086	0.063	0.020	0.013	0.028
	29		0.059	0.055	0.027	0.039	0.025
	29.2	0.023				0.007	0.025
	30	0.063	0.033	0.055	0.020	0.010	0.025

Table 9 (Continued) Allele frequencies, statistical parameters, and references of DXS10011 in six populations. For the Thai population, the data were from pooled males and females (*Table 3*) when the exact tests showed no difference between the two groups. No significant most common allele shared in any population was observed. Some statistical parameters were not available.

Parameter		Thai	Japan	Taiwan	Latvia	German	Algeria
Allele (Continued)	30.1	0.023	0.007				
	30.2				0.081	0.030	0.044
	31	0.018	0.026	0.068	0.007	0.010	0.038
	31.2	0.008			0.095	0.070	0.047
	31.3	0.028	0.007				
	32		0.046	0.047	0.027	0.050	
	32.1	0.015	0.007				
	32.2				0.074	0.075	
	33	0.040	0.039	0.055		0.023	
	33.1	0.033	0.007				
	33.2				0.054	0.059	0.025
	34	0.065	0.033	0.041	0.041	0.043	
	34.1	0.010	0.007				
	34.2				0.014	0.023	
	35	0.050	0.053	0.027	0.068	0.033	0.044
	35.2	0.025			0.027	0.013	0.013
	36	0.065	0.013	0.030	0.074	0.075	0.073
	36.2	0.063				0.003	0.003
	37		0.013	0.008	0.047	0.059	0.079
	37.2	0.005					0.003
	38	0.098	0.020	0.011	0.088	0.089	0.070
	39	0.053		0.005	0.034	0.010	0.054
	39.1	0.055					0.003
	40		0.007	0.008	0.068	0.053	0.063

Table 9 (Continued) Allele frequencies, statistical parameters, and references of DXS10011 in six populations. For the Thai population, the data were from pooled males and females (*Table 3*) when the exact tests showed no difference between the two groups. No significant most common allele shared in any population was observed. Some statistical parameters were not available.

Parameter		Thai	Japan	Taiwan	Latvia	German	Algeria
Allele (Continued)	41	0.045		0.003	0.027	0.056	
	42	0.063			0.034	0.046	
	43	0.030			0.014	0.053	
	44	0.033		0.003	0.020	0.023	
	45	0.025			0.020	0.016	
	46	0.023			0.014	0.007	
	47	0.005			0.007	0.007	
	48	0.005					
	49	0.010					
	50	0.003					
	51	0.005					
	PDm	0.946	0.933	—	0.942	0.948	—
	PDf	0.979	0.997	—	0.994	0.995	—
	MEC II/III	0.946	0.957	—	0.939	0.945	—
	MEC IV	0.901	—	—	0.889	0.899	—
Reference		—	(19)	(21)	(24)	(15)	(26)

Note: *MEC = Mean exclusion chance (II for X-chromosomal markers in trios; III for X-chromosomal markers in trios; and IV for X-chromosomal markers in father/daughter pairs); PDf = Power of discrimination in females; and PDm = Power of discrimination in males*

DXS10011 had a very low HETobs (0.670) compared to the expected heterozygosity (HETexp) (0.950) as a result of an amplification difficulty, leading to a genotypic error (homozygote excess)[12]. It also had the largest product size compared to DXS8378, DXS101, HPRTB, and DXS8377[4,13-15]. In addition, DXS10011 contained a highly polymorphic allele, mainly because of complex structural variants (regular and inter-alleles)[14]. We found that the shorter fragment size amplified better than the larger fragment which produced allelic drop-out, known as extreme preferential amplification (EPA). Due to a difficulty to distinguish the longer allele from background noise, the overrepresentation of homozygotes for the shorter allele could occur[12]. Therefore, some samples were repeatedly investigated.

Because the recombination rate of the X chromosome in females is low, the X chromosome has greater linkage disequilibrium (LD) as compared to autosomal markers. The exact test for LD was performed for all pairs of the loci in this study, the result showed no LD in each pair of markers. In the kinship cases involving 15 family trios with daughter, no mutation was detected. This proved the applicable of five X-STR markers in kinship cases.

Conclusions

This five X-chromosomal STRs multiplex system offered sufficient polymorphic patterns in one single reaction. It worked with reasonable amounts of DNA, suitable for forensic casework, and yielded reproducible results. It could be recommended for routine paternity analysis in complex deficiency cases or for a complement of autosomal and Y-STRs analysis.

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References

(1). B. Brinkmann, Overview of PCR-based systems in identity testing, Methods Mol. Biol. 198 (1998) 105–119.

(2). M. Kayser, A. Caglia´, D. Corach, N. Fretwell, C. Gehrig, G. Graziosi, F. Heidorn, S. Herrmann, B. Herzog, M. Hidding, K. Honda, M. Jobling, M. Krawczak, K. Leim, S. Meuser, E. Meyer, W. Oesterreich, A. Pandya,W. Parson, G. Penacino, A. Perez-Lezaun, A. Piccinini, M. Prinz, C. Schmitt, P.M. Schneider, R. Szibor, J. Teifel-Greding, G.Weichhold, P. de Knijff, L. Roewer, Evaluation of Y-chromosomal STR's: a multicenter study, Int.J.Legal Med. 110 (1997) 125–133.

(3). J. Edelmann, R. Szibor, Validation of the HumDXS6807 short tandem repeat polymorphism for forensic application, Electrophoresis 20 (1999) 2844–2846.

(4). J. Edelmann, R. Szibor, DXS101: a highly polymorphic X-linked STR, Int. J. Legal Med. 114 (2001) 301–304.

(5). R. Szibor, M. Krawczak, S. Hering, J. Edelmann, E. Kuhlisch,D. Krause, Use of X-linked markers for forensic purposes, Int.J.Legal Med. 117 (2003) 67–74.

(6). S. Turrina, D. De Leo, Population data of three X-chromosomal STRs: DXS7132, DXS7133 and GATA172D05 in North Italy, J. Forensic Sci. 48 (2003) 1428–1429.

(7). M. Lv, W. Liang, M. Wu, M. Liao, B. Zhou, Y. Jia, L. Zhang, Allele frequency distribution of two X-chromosomal STR loci in Han population in China, J. Forensic Sci. 49 (2004) 418–419.

(8). M. Ross, D. Grafham, A. Coffey, The DNA sequence of the human X chromosome, Nature 434 (2005) 325–337.

(9). R.H. Don, P.T. Cox, B.J. Wainwright, K. Baker, J.S. Mattick, ''Touch-down'' PCR to circumvent spurious priming during gene amplification, Nucleic Acids Res. 19 (1991) 4008.

(10). DNA Commission of the International Society of Forensic Hemogenetics, DNA recommendations-further report of the DNA commission of the ISFH regarding the use of short tandem repeat systems, Int.J.Legal Med 110 (1997) 175–176.

(11). R. Fisher, Standard calculations for evaluating a blood group system, Heredity 5 (1951) 95–102.

(12). J.J. Chen, T. Duan, R. Single, K. Mather, G. Thomson, Hardy-Weinberg testing of a single homozygous genotype, Genetics 170 (2005) 1439-1442.

(13). S. Hering, N. Brundirs, E. Kuhlisch, J.Edelmann, I. Plate, M. Benecke, P.H. Van, M. Micheal, R.Szibor, DXS10011: studies on structure, allele distribution in three populations and

genetic linkage to further q-telomeric chromosome X markers, Int.J.Legal Med 118 (2004) 313-319.

(14). J. Edelmann, D. Deichsel, S. Hering, I. Plate, R. Szibor, Sequence variation and allele nomenclature for the X-linked STRs DXS9895, DXS8378, DXS7132, DXS6800, DXS7133, GATA172D05, DXS7423 and DXS8377, Forensic Sci.Int.129 (2002) 99-103.

(15). M. Poetsch, H. Petersmann, A. Repenning, E. Lignitz, Development of two pentaplex systems with X-chromosomal STR loci and their allele frequencies in a northeast German population, Forensic Sci.Int. 155 (2005) 71-76.

(16). D. Athanasiadou, B. Stradmann-Bellinghausen, C. Rittner, K.W. Alt, P.M. Schneider, Development of a quadruplex PCR system for the genetic analysis of X-chromosomal loci, Int. Congress Series 1239 (2003) 311-314.

(17). H. Asamura, H. Sakai, M. Ota, M. Fukushima, Japanese population data for eight X-STR loci using two new quadruplex systems, Int J Legal Med. 120 (2006) 303–309.

(18). H. Asamura,H. Sakai,K. Kobayashi,M. Ota, M. Fukushima, MiniX-STR multiplex system population study in Japan and application to degraded DNA analysis, Int.J.Legal Med. 120 (2006) 174–181.

(19). A. Tamura, M. Iwata, I. Takase, T. Miyazaki, K. Matsui, H. Nishio, K. Suzuki, Analysis of two types of novel alleles in the DXS10011 locus, Legal Medicine. 6 (2004) 52–54.

(20). W.M. Tang, K.Y.To, Four X-chromosomal STRs and their allele frequencies in a Chinese population, Forensic Sci.Int. 162 (2006) 64–65.

(21). M. Chen, C. Pu, Population data on the X chromosome short tandem repeat loci DXS10011, DXS101, DXS6789, DXS7132, DXS8377and DXS9895 in Taiwan, Forensic Sci.Int. 146 (2004) 65–67.

(22). S. Turrina, R. Atzei, G. Filippini, D. De Leo, Development and forensic validation of a new multiplex PCR assay with 12 X-chromosomal short tandem repeats, Forensic Sci.Int.: Genetics. 1 (2007) 201–204.

(23). C. Robino, A. Giolitti, S. Gino, C. Torre, Analysis of 12 X-chromosomal short tandem repeats in the North-West Italian population by means of two multiplex PCRs, Int. Congress Series. 1288 (2006) 316–318.

(24). M. Poetsch, A. Sabule, H. Petersmann, V. Volksone, E. Lignitz, Population data of 10 X-chromosomal loci in Latvia, Forensic Sci.Int. 157 (2006) 206–209.

(25). I. Gomes, C. Alves, K. Maxzud, R. Pereira, M.J. Prata, P. Sa´nchez-Diz, A. Carracedo, A. Amorim, L. Gusma˜o, Analysis of 10 X-STRs in three African populations, Forensic Sci.Int.: Genetics. 1 (2007) 208–211.

(26). A. Bekada, S. Benhamamouch, A. Boudjema, M. Fodil, S. Menegon, C. Torre, C. Robino, Analysis of 12 X-chromosomal STRs in an Algerian population sample, Forensic Sci.Int.: Genetics Supplement Series. 2 (2009) 400–401.

ORIGINAL ARTICLE

The detection of HLA-B27 allele types by quantitative real-time polymerase chain reaction with melting curve analysis

Supichaya Nimnuan-ngam[1], Pasra Arnutti[2*] and Thirayost Nimmanon[2]

[1] *Siriraj Center of Research of Excellence in Immunoregulation, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

[2] *Department of Pathology, Phramongkutklao College of Medicine, Bangkok, Thailand*

* Correspondence to: Colonel Assistant Professor Dr Pasra Arnutti, Department of Pathology, Floor 6, Her Royal Highness Princess Bejaratana Building, Phramongkutklao College of Medicine, 317 Rajavithi Road, Rajadevi, Bangkok 10400 Thailand. Telephone: +66 (0) 83 619 8689 Fax: +66 (0) 2 354 7791 Email: pasra@pcm.ac.th, pasra@pcmpathology.org, pasra@hotmail.com

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Abstract

Human leucocyte antigen B27 (HLA-B27) is a major histocompatibility complex class 1 molecule that is strongly associated with ankylosing spondylitis. The polymerase chain reaction with sequence specific primers (PCR-SSP) is currently used for the discrimination of HLA-B27 allele types resulting from single base substitutions. However, this technique is a multi-step, time-consuming task and increases the chance of post-PCR contamination. The main objective of this study is to determine the HLA-B27 genotypes using quantitative real-time polymerase chain reaction (Q-PCR) followed by melting curve analysis. Genomic DNA (gDNA) was isolated from 50 outdated peripheral blood samples stored at -20 °C in the Molecular Genetics Laboratory, Department of Pathology, Phramongkutklao College of Medicine, Bangkok, Thailand. These blood specimens were previously collected from 25 patients each who were HLA-B27 negative and positive in PCR-SSP. The genotyping of HLA-B27 was detected in the isolated gDNA by a Q-PCR assay with melting curve analysis. All HLA-B27-positive blood samples yielded either a single peak or double peaks at 87.28 ± 1.36 °C. Only one peak for an endogenous reference gene (human *beta-globin* gene) was detectable at 84.90 ± 0.09 °C in all HLA-B27-negative blood samples. The Q-PCR followed by melting curve analysis is reliable for the DNA typing of the HLA-B27 alleles and could be used to an alternative to a conventional PCR-SSP method.

Keywords: human leucocyte antigen B27; melting curve analysis; quantitative real- time polymerase chain reaction

Introduction

Ankylosing spondylitis (AS) is a form of arthritis that effects the spine[1]. It causes inflammation of the spinal joints that can lead to severe and chronic pain. Human leukocyte antigen B27 (HLA-B27) is associated with AS, including ocular diseases, rheumatic diseases and inflammatory bowel disease. Approximately 95% of patients with AS have HLA-B27 compared with only 8% of healthy individuals. Several methods have been developed for the identification of the HLA-B27 allele[1], i.e. serology, polymerase chain reaction (PCR), including the standard PCR with sequence-specific primers (SSP). However, PCR is a time-consuming process and also requires post-PCR manual procedures[2]. Currently, the application of real-time PCR or quantitative PCR (qPCR) with a double-stranded DNA binding SYBR Green dye has facilitated the rapid detection and amplification of PCR products[3,4]. The aim of this study was to develop of the qPCR analysis for HLA-B27 allele test in routine work.

Materials and Methods

Samples:

Fifty outdated peripheral blood samples were enrolled in this study. They are comprised of 25 HLA-B27-negative and 25 HLA-B27-positive blood samples. All blood samples were kept at -20 °C until DNA isolation and analysis[5]. Genomic DNA was isolated from 500 µL of whole blood using the AxyPrep™ Blood Genomic DNA Miniprep Kit (CA, USA) according to the manufacturer's instructions.

Real-time PCR analysis:

The real-time PCR was performed on qTower^3-Real-Time PCR thermocycler (Analytik Jena AG, Germany). A total volume of 12 µL of each mixture consisted of 5 µl of SYBR Green I (EXPRESS SYBR™ GreenER™ qPCR Supermix, universal; Invitrogen, USA); 4 µL of detection mix containing the primer E91S and 136AS specific for HLA-B27, primer BG1 and BG2 specific for ß-globin[3]; 1 µL of distilled water; and 2 µl of DNA template. The real-time PCR amplification protocol for this reaction consisted of an initial denaturation step at 95 °C for 5 sec, and 45 cycles of 95 °C for 4 sec, 68 °C for 1 min, 95 °C for 45 sec, 58 °C for 1 sec and final extension at 72 °C for 33 sec. After amplification was complete, a final melting curve analysis (T_m) was followed by the generation of a thermal gradient from 60 °C to 95 °C with a ramp rate of 5 °C/s. HLA-B27 positive samples give a unique melting peak at 87.28 ± 1.36 °C. Therefore, HLA-B27 negative samples showed a single temperature curve at 84.90 ± 0.09 °C representing the ß-globin[5,6].

The results were confirmed on the agarose gel. The melting peak for ß-globin showed 268 bp PCR product and the melting peaks for HLA-B27 alleles showed 136 bp PCR product representative of HLA-B27-positive samples[5].

Results

The results of real-time PCR showed that among 50 samples, 25 samples were HLA-B27-positive and 25 samples were HLA-B27-negative. All negative samples had only one peak at 84.90 ± 0.09 ℃ (*Figure 1A*). All positive samples showed single or double peaks at 87.28 ± 1.36 ℃ (*Figure 1B*). This signal was absent for the HLA-B27 negative samples. The melting peaks for ß-globin (T_m = 84.90 ± 0.09 ℃) and the 268 bp PCR product were visible on the agarose gel correlated with HLA-B27-negative samples (*Figure 2A*). The melting peaks for HLA-B27 (T_m = 87.28 ± 1.36 ℃) and the 136 bp PCR product are visible on the agarose gel correlated with HLA-B27-positive samples (*Figure 2B*). Despite the lower fragment length, the HLA-B27 specific PCR product (136 bp) yielded a higher T_m value compared with the ß-globin 268 bp product because of a higher GC content[5].

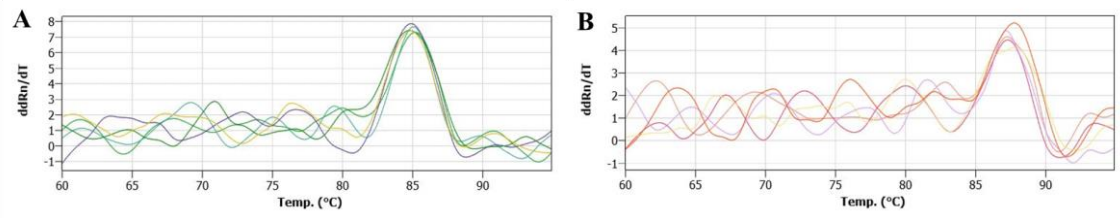

Figure 1 Melting curve analysis (T_m) from HLA-B27 negative samples (A) and HLA-B27 positive samples (B). The T_m of negative samples at 84.90 ± 0.09 ℃ results from the ß-globin. The T_m of positive samples at 87.28 ± 1.36 ℃ results from the HLA-B27.

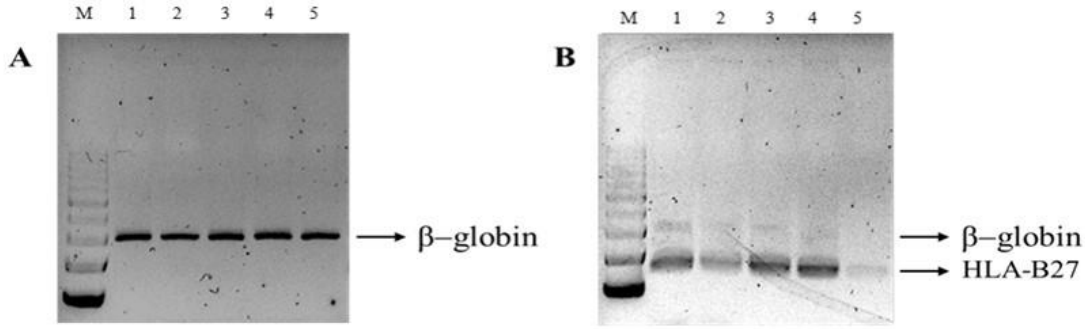

Figure 2 Gel electrophoresis on 2% agarose of PCR products from after real-time PCR. PCR products of HLA-B27 using E91S, 136AS primers and β-globin. The 268 bp PCR product specific for HLA-B27 negative samples **(A)**. The 136 bp PCR product specific for HLA-B27 positive samples **(B)**. Lane M, DNA ladder ranges from 100 to 1000 bp.

Discussions

We compared the results of 50 genotyped individual with our previous original PCR method. All genotypes were completely concordant. However, conventional PCR requires post-PCR manipulations that increase the risk of cross-contamination between samples. These post-PCR steps are laborious, especially when large numbers of samples and cost-effective[6,7]. Thus, we are starting point for development of a real-time PCR. Real-time PCR is one of the methods used to detect for HLA-B27 allele in patients suspected to have AS and related diseases. Real-time PCR is much more accurate than PCR. Real-time PCR substantially reduced the labor-intensive steps and the total processing time when compared to the usual time required for PCR. It is therefore a suitable in routine laboratory practice[8].

Conclusion

The real-time PCR assay is possibly reliable for the detection of HLA-B27.

References

(1). Eun Hae Cho MD, Sang Gon Lee MD, Jeong Ho Seok MD, Bo Ya Na Park MS, Eun Hee Lee MD. Evaluation of Two Commercial HLA-B27 Real-Time PCR Kits. Korean J Lab Med. 2009;29:589-93.

(2). Nätterkvist Y. Development of a PCR method to detect HLA-B27 in ankylosing spondylitis. Department of Medical Biochemistry and Microbiology. 2012;15:1-22.

(3). Tiemann C, Vogel A, Dufaux B, Zimmer M, Krone J-R, Hagedorn H-J. Rapid DNA Typing of HLA-B27 Allele By Real-Time PCR Using LightCycler Technology. Clin Lab. 2001;47:131-4.

(4). Gersuk VH, Nepom GT. A real-time PCR approach for rapid high resolution subtyping of HLA-DRB1*04. J Immunol Methods. 2007;317:1-11.

(5). Bon MAM, Oeveren-Dybicz Av, Bergh FAJTMvd. Genotyping of HLA-B27 by Real-Time PCR without Hybridization Probes. Clinical Chemistry. 2000;7:1000-2.

(6). Faner R, Casamitjana N, Colobran R, Ribera A, Pujol-Borrell R, Palou E, et al. HLA-B27 Genotyping by Fluorescent Resonance Emission Transfer (FRET) Probes in Real-Time PCR. Human Immunology. 2004;65:826-38.

(7). Panichrum P, Chuesakul M, Fungkajai M, Kaset C, Nathalang O. HLA-B27 Screening Using PCR-SSP Technique in Thai Blood Donors. J Med Tech Assoc Thailand. 2013;41:4440-5.

(8). Casamitjana N, Faner R, Santamaria A, Colobran R, Ribera A, Pujol-Borrell R, et al. Development of a New HLA-DRB Real-Time PCR Typing Method. Human Immunology. 2005;66:85-91.

APPENDIX 1
INFORMATION FOR AUTHORS

All authors listed in a paper submitted to Asian Archives of Pathology (AAP) must have contributed substantially to the work. It is the corresponding author who takes responsibility for obtaining permission from all co-authors for the submission. When submitting the paper, the corresponding author is encouraged to indicate the specific contributions of all authors (the author statement, with signatures from all authors and percentage of each contribution can be accepted). Examples of contributions include: designed research, performed research, contributed vital new reagents or analytical tools, analysed data, and wrote the paper. An author may list more than one type of contribution, and more than one author may have contributed to the same aspect of the work.

Authors should take care to exclude overlap and duplication in papers dealing with related materials. See also paragraph on Redundant or Duplicate Publication in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" at http://www.icmje.org/index.html.

The submitted manuscripts will be reviewed by the members of the Editorial Board or the expert reviewers. At the discretion of the Editorial Board, the manuscripts may be returned immediately without full review, if deemed not competitive or outside the realm of interests of the majority of the readership of the Journal. The decision (reject, invite revision, and accept) letter will be coming from the Editorial Board who has assumed responsibility for the manuscript's review. The editor's decision is based not just on technical merit of the work, but also on other factors such as the priority for publication and the relevance to the Journal's general readership. All papers are judged in relation to other submissions currently under consideration.

Categories of Manuscripts

1. Letters to the Editor

The letters to the editor are the reactions to any papers published in AAP. These letters will be reviewed by the Editorial Board and sent to the authors of the original paper with an invitation to respond. Letters and eventual responses will be published together, when appropriate.

- *Word Count: 300 – 500 words (excluding references and figure or table legends)*
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The original articles are the researches describing the novel understanding of anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. Systematic reviews, meta-analyses and clinical trials are classified as articles. The articles should be clearly and concisely written in the well-organised form (see ***Organisation of Manuscripts***): abstract; introduction; materials and methods; results; discussion; and conclusions. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two or more experts in the field.

- *Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)*
- *Structured Abstract (see* ***Organisation of Manuscripts****): 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 6*

3. Review Articles

The review articles are generally invited by the Editor-in-Chief. They should focus on a topic of broad scientific interest and on recent advances. These articles are peer-reviewed before the final decision to accept or reject the manuscript for publication. Therefore, revisions may be required.

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- *References: Maximum of 150*
- *Figures or Tables: Maximum of 4*

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AAP limits publication of case reports to those that are truly novel, unexpected or unusual, provide new information about anatomical pathology, clinical pathology (laboratory medicine) or forensic medicine (legal medicine or medical jurisprudence). In addition, they must have educational value for the aforementioned fields. The journal will not consider case reports describing preventive or therapeutic interventions, as these generally require stronger evidence. Case reports that involve a substantial literature review should be submitted as a review article. The submitted case reports will undergo the usual peer-reviewed process.

- *Word Count: 1,200 – 2,000 words (excluding abstract, references, and figure or table legends)*
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- *References: Maximum of 20*
- *Figures or Tables: Maximum of 4*

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Case illustrations are aimed to provide education to readers through multidisciplinary clinicopathological discussions of interesting cases. The manuscript consists of a clinical presentation or description, laboratory investigations, discussion, final diagnosis, and up to 5 take-home messages (learning points). Regarding continuous learning through self-assessment, each of the case illustrations will contain 3 – 5 multiple choice questions (MCQs) with 4 – 5 suggested answers for each question. These MCQs are placed after the final diagnosis and the correct answers should be revealed after the references. The questions and take-home messages (learning points) are included in the total word count. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two experts in the field.

- *Word Count: 1,000 – 2,000 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 10*
- *Figures: Maximum of 2*
- *Tables: Maximum of 5*

6. Technical Notes

The technical notes are brief descriptions of scientific techniques used in the anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. The submitted manuscripts are usually peer-reviewed.

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Organisation of Manuscripts

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The manuscripts written in English language are preferable. However, Thai papers are also acceptable, but their title pages, abstracts, and keywords must contain both Thai and English. These English and Thai manuscripts are prepared in A4-sized Microsoft Word documents with leaving 2.54-cm (1-inch) margins on all sides. All documents are required to be aligned left and double-spaced throughout the entire manuscript. The text should be typed in 12-point regular Times New Roman font for English manuscript and 16-point regular TH SarabunPSK font for Thai manuscript.

The running titles of English and Thai manuscripts are placed in the top left-hand corner of each page. They cannot exceed 50 characters, including spaces between words and punctuation. For the header of English paper, the running title will be typed in all capital letters. The page number goes on the top right-hand corner.

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All studies that involve human subjects should not mention subjects' identifying information (e.g. initials) unless the information is essential for scientific purposes and the patients (or parents or guardians) give written informed consent for publication.

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The title page is the first page of the manuscripts and must contain the following:

- The title of the paper (not more than 150 characters, including spaces between words)
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- The name, surname, full postal address, telephone number, facsimile number, and email address of the corresponding author who will take primary responsibility for communication with AAP.
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3. Abstract

A structured form of abstract is used in all Original Article manuscripts and must include the following separate sections:

- *Background: The main context of the study*
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- *Materials and Methods: How the study was performed*
- *Results: The main findings*
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- *<u>Keywords</u>: 3 – 5 words or phrases (listed in alphabetical order) representing the main content of the article*

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The Introduction section should clearly explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

5. Materials and Methods

The Materials and Methods section must be described in sufficient detail to allow the experiments or data collection to be reproduced by others. Common routine methods that have been published in detail elsewhere should not be described in detail. They need only be described in outline with an appropriate reference to a full description. Authors should provide the names of the manufacturers and their locations for any specifically named medical equipment and instruments, and all chemicals and drugs should be identified by their systematic and pharmaceutical names, and by their trivial and trade names if relevant, respectively. Calculations and the statistical methods employed must be described in this section.

All studies involving animal or human subjects must abide by the rules of the appropriate Internal Review Board and the tenets of the recently revised Helsinki protocol. Hence, the manuscripts must include the name of the ethics committee that approved the study and the committee's reference number if appropriate.

6. Results

The Results section should concisely describe the findings of the study including, if appropriate, results of statistical analysis which must be presented either in the text or as tables and figures. It should follow a logical sequence. However, the description of results should not simply repeat the data that appear in tables and figures and, likewise, the same data should not be displayed in both tables and figures. Any chemical equations, structural formulas or mathematical equations should be placed between successive lines of text. The authors do not discuss the results or draw any conclusions in this section.

7. Discussion

The Discussion section should focus on the interpretation and the significance of the findings against the background of existing knowledge. The discussion should not repeat information in the results. The authors will clearly identify any aspects that are novel. In addition, there is the relation between the results and other work in the area.

8. Conclusions

The Conclusions section should state clearly the main summaries and provide an explanation of the importance and relevance of the study reported. The author will also describe some indication of the direction future research should take.

9. Acknowledgements

The Acknowledgements section should be any brief notes of thanks to the following:

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- *A person who provided purely technical help or writing assistance*
- *A department chair who provided only general support*
- *Sources of material (e.g. novel drugs) not available commercially*

Thanks to anonymous reviewers are not allowed. If you do not have anyone to acknowledge, please write "Not applicable" in this section.

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The Vancouver system of referencing should be used in the manuscripts. References should be cited numerically in the order they appear in the text. The authors should identify references in text, tables, and legends by Arabic numerals in parentheses or as superscripts. Please give names of all authors and editors. The references should be numbered and listed in order of appearance in the text. The names of all authors are cited when there are six or fewer. When there are seven or more, only the first three followed by "et al." should be given. The names of journals should be abbreviated in the style used in Index Medicus (see examples below). Reference to unpublished data and personal communications should not appear in the list but should be cited in the text only (e.g. A Smith, unpubl. Data, 2000).

- *Journal article*

  1. Sibai BM. Magnesium sulfate is the ideal anticonvulsant in preeclampsia – eclampsia. Am J Obstet Gynecol 1990; 162: 1141 – 5.

- *Books*

  2. Remington JS, Swartz MN. Current Topics in Infectious Diseases, Vol 21. Boston: Blackwell Science Publication, 2001.

- *Chapter in a book*

  3. Cunningham FG, Hauth JC, Leveno KJ, Gilstrap L III, Bloom SL, Wenstrom KD. Hypertensive disorders in pregnancy. In: Cunningham FG, Hauth JC, Leveno KJ, Gilstrap L III, Brom SL, Wenstrom KD, eds. Williams Obstetrics, 22nd ed. New York: McGraw-Hill, 2005: 761 – 808.

11. Tables

The tables should be self-contained and complement, but without duplication, information contained in the text. They should be numbered consecutively in Arabic numerals (Table 1, Table 2, etc.). Each table should be presented on a separate page with a comprehensive but concise legend above the table. The tables should be double-spaced and vertical lines should not be used to separate the columns. The column headings should be brief, with units of measurement in parentheses. All abbreviations should be defined in footnotes. The tables and their legends and footnotes should be understandable without reference to the text. The authors should ensure that the data in the tables are consistent with those cited in the relevant places in the text, totals add up correctly, and percentages have been calculated correctly.

12. Figure Legends

The legends should be self-explanatory and typed on a separate page titled “Figure Legends”. They should incorporate definitions of any symbols used and all abbreviations and units of measurement should be explained so that the figures and their legends are understandable without reference to the text.

If the tables or figures have been published before, the authors must obtain written permission to reproduce the materials in both print and electronic formats from the copyright owner and submit them with the manuscripts. These also follow for quotes,

illustrations, and other materials taken from previously published works not in the public domain. The original resources should be cited in the figure captions or table footnotes.

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All illustrations (line drawings and photographs) are classified as figures. The figures should be numbered consecutively in Arabic numerals (Figure 1, Figure 2, etc.). They are submitted electronically along with the manuscripts. These figures should be referred to specifically in the text of the papers but should not be embedded within the text. The following information must be stated to each microscopic image: staining method, magnification (especially for electron micrograph), and numerical aperture of the objective lens. The authors are encouraged to use digital images (at least 300 d.p.i.) in .jpg or .tif formats. The use of three-dimensional histograms is strongly discouraged when the addition of these histograms gives no extra information.

14. Components

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The Letter to the Editor manuscripts consist of the following order:

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- *Figure (if needed)*

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- *Conclusions*

- *Acknowledgements*
- *References*
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- *Table (s)*
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- *References*
- *Correct Answers to MCQs*
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- *References*
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- *Figure (s)*

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The authors of the accepted manuscripts will receive proofs and are responsible for proofreading and checking the entire article, including tables, figures, and references. These authors should correct only typesetting errors at this stage and may be charged for extensive alterations. Page proofs must be returned within 48 hours to avoid delays in publication.

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Phramongkutklao College of Medicine

317 Rajavithi Road, Rajadevi, Bangkok 10400 Thailand

Telephone: +66 (0) 90 132 2047

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Editor-in-Chief of Asian Archives of Pathology

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Dr Chetana Ruangpratheep

The Editorial Office of Asian Archives of Pathology

Department of Pathology, Floor 6, Her Royal Highness Princess Bejaratana Building

Phramongkutklao College of Medicine

317 Rajavithi Road, Rajadevi, Bangkok 10400 Thailand

Telephone: +66 (0) 90 132 2047

Fax: +66 (0) 2 354 7791

Email: editor@asianarchpath.com

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