

A. 4 HLA and disease	12
B. Graves' disease	13
B. 1 Etiology and pathogenesis	14
B. 2 HLA antigen associated with Graves' disease	16
III. MATERIALS AND METHODS	20
A. Subjects	20
B. Preparation of genomic DNA	21
C. DNA amplification	22
C. 1 PCR primer pairs for amplification	22
C. 2 Polymerase chain reaction	22
D. Dot blot hybridization	22
D. 1 Dot spotting	22
D. 2 Labeling of the oligonucleotide probes	23
D. 2.1 Sequence-specific oligonucleotide (SSO) probes	23
D. 2.1 3'-end labeling with DIG-ddUTP	23
D. 2.3 Hybridization	23
E. Chemiluminescent detection	24
F. Dehybridization	24
G. Interpretation of the results and statistical analysis	25
G. 1 Grading of the chemiluminescent signal	25
G. 2 Statistical analysis	26
G. 2.1 Relative risk	26
G. 2.2 Chi square test	26
G. 2.3 Etiologic fraction and preventive fraction	27
G. 2.4 Linkage disequilibrium	29
H. The new HLA-DRB1, HLA-DQA1 and HLA-DQB1 alleles and new probes	30

IV. RESULTS	45
A. Identification of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles by hybridization and non-isotypic detection	45
A. 1 HLA-DQA1 locus	45
A. 2 HLA-DQB1 locus	45
A. 3 HLA-DRB1 locus	45
A. 4 Other group of the HLA-DRB1 locus	46
A. 4.1 DR1 and DR4 groups	46
A. 4.2 DR2 group	47
A. 4.3 DR52 associated DRB1 group	47
B. Distribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles in male and female Graves' patients	47
B. 1 HLA-DQA1 locus	47
B. 2 HLA-DQB1 locus	48
B. 3 HLA-DRB1 locus	48
C. Distribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles among patients with exophthalmos	49
D. Distribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles among patients with relapse/exacerbation	50
E. Distribution of HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles among patients with uncommon manifestations	50
F. Comparison of the frequency of polymorphic amino acid residues in HLA-DQA1 and DQB1 molecules	51
G. Distribution of HLA class II haplotypes in Graves' patients	52
V. DISCUSSION	79
VI. SUMMARY	92

VII. REFERENCES	94
VIII. APPENDIX	109
IX. CURRICULUM VITAE	113

มหาวิทยาลัยเชียงใหม่
Chiang Mai University

LIST OF TABLES

TABLE		PAGE
1	HLA antigens and alleles significantly associated with a high risk for Graves' disease in representative studies	18
2	The new alleles of the HLA-DQA1, HLA-DQB1 and HLA-DRB1 genes, update June 1995	31
3	HLA-DQA1, HLA-DQB1, HLA-DRB1 generic and group specific primer pairs	33
4	Conditions of PCR for HLA-DQA1, HLA-DQB1, HLA-DRB1 generic and HLA-DRB1 group specific typing	34
5	Sequences and specificities of DQA SSO probes	35
6	Sequences and specificities of DQB SSO probes	36
7	Sequences and specificities of DRB SSO probes for HLA-DRB1 generic group typing	37
8	Sequences and specificities of DRB SSO probes for HLA-DR52 associated DRB1 typing	38
9	Sequences and specificities of DRB SSO probes for HLA-DR2 typing	39
10	Hybridization pattern of DQA SSO probes for HLA-DQA1 typing	40
11	Hybridization pattern of DQB SSO probes for HLA-DQB1 typing	41
12	Hybridization pattern of DRB SSO probes for HLA-DRB1 typing	42
13	Hybridization pattern of DRB SSO probes for HLA-DR52 associated DRB1 typing	43
14	Hybridization pattern of DRB SSO probes for HLA-DR2 typing	44
15	The serological specificity corresponded to HLA-DRB1 alleles	53
16	The antigen frequency of HLA-DQA1 gene of Graves' male and female patients comparing with normal control subjects	54

20	The antigen frequency of HLA-DRB1 gene between patients with Graves' disease and normal controls	58
21	The allele frequency of HLA-DRB1 gene between Graves' male and female patients compared with normal controls	59
22	The antigen and allele frequencies of HLA-DQA1 locus of Graves' patients with and without exophthalmos	60
23	The antigen frequency of HLA-DQB1 alleles of Graves' patients with and without exophthalmos	61
24	The allele frequency of HLA-DQB1 alleles of patients with and without exophthalmos	62
25	The antigen frequency of HLA-DRB1 locus of patients with Graves' patients with and without exophthalmos	63
26	The allele frequency of HLA-DRB1 alleles of patients with Graves' disease with and without exophthalmos	64
27	The antigen frequency of HLA-DR serological specificity of patients with Graves' disease	65
28	The allele frequency of HLA-DR depending on the serological specificity of patients with Graves' disease	66
29	The allele frequency of HLA-DQA1 alleles of patients with Graves' disease with relapse/exacerbation	67
30	The allele frequency of HLA-DQB1 alleles of patients with Graves' disease with relapse/exacerbation	68
31	The allele frequency of HLA-DRB1 alleles of patients with Graves' disease with relapse/exacerbation	69
32	The allele frequency of HLA-DQA1 gene of Graves' patients belonging to the age of onset	70
33	The distribution of the HLA-DQB1 alleles of patients with Graves' disease according to the age of onset of disease	71
34	The distribution of the HLA-DQA1 alleles of patients with Graves' disease with and without exophthalmos according to the age of onset of disease	72
35	The distribution of the HLA-DQB1 alleles belonging to the age of onset of disease among patients with and without exophthalmos	73
36	The distribution of the HLA-DQA1 alleles of patients with Graves' disease with relapse/exacerbation according to the age of onset of disease	74

37	The distribution of the HLA-DQB1 alleles according to the age of onset among patients with relapse/exacerbation	75
38	The antigen and allele frequencies of Graves' patients with uncommon manifestations	76
39	The linkage disequilibrium of HLA-DRB1-DQA1, HLA-DRB1-DQB1 and HLA-DQB1-DQA1 which were presented by haplotype frequencies and delta	77
40	The haplotype frequencies of HLA-DRB1-DQA1-DQB1 haplotypes of Graves' patients compared with those of normal control from Chiang Mai and Bangkok	78

LIST OF ILLUSTRATIONS

FIGURE		PAGE
1	Map of the human major hitocompatibility complex	7
2	Genomic organization of the HLA-DR region and encoded products	8
3	Map of HLA-DR1/HA peptide contracts	19
4	The age of onset of disease of Graves' patients separated by gender	32

ABBREVIATIONS

Allele freq.	Allele frequency
Ag. freq.	Antigen frequency
β_2 M	Beta-2 microglobulin
BSA	Bovine serum albumin
CIA	Collagen-induced arthritis
CLIP	Class II associated invariant chain peptide
cm	centimeter
$^{\circ}$ C	degree celcius
CSPD	Disodium3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo [3.3.1.1.1.]decan}-4-yl)phenyl phosphate
CTLA-4	Cytotoxic T lymphocyte antigen-4
DIG-ddUTP	Digoxigenin dideoxyuridine-triphosphate
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
TTP	Thymidine triphosphate
EDTA	Ethylene diaminetetraacetic acid
ELAM-1	Endothelial leukocyte adhesion molecule-1
EF	Etiologic fraction
HA	Hemagglutinin
HLA	Human leukocyte antigen
ICAM-1	Intracellular adhesion molecule-1
IDDM	Insulin dependent diabetes mellitus
IDR	Incidence density ratio
LFA-1	Lymphocyte function associated antigen-1

MHC	Major histocompatibility complex
μl	micrometer
nm	nanometer
ND	Not done
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PF	Preventive fraction
PVP	Polyvinylprolidone
RA	Rheumatoid arthritis
RFLP	Restriction-fragment length polymorphism
rpm	round per minute
RR	Relative risk
SSO	Sequence-specific oligonucleotide
SDS	Sodium dodecyl sulfate
TSAb	Thyroid stimulating antibody
TAE	Tris/acetate/EDTA
TMAC	Tetramethylammonium chloride
TSH	Thyroid stimulating hormone
TSHR	Thyroid stimulating hormone receptor
VCAM-1	Vascular cell adhesion molecule-1
VLA-1	Very late activation antigen-1