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Comprehensive analysis of seven *Toll-like receptor* genes including 15 single-nucleotide polymorphisms with autoimmune thyroid disease in Korean children.

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Background: **The *Toll-like receptors (TLRs)* are germline-encoded receptors that play an essential role in initiating the immune response against pathogens.**

Objective and hypotheses: **In this study, we assess the association of *TLR* polymorphism with autoimmune thyroid disease (AITD) in Korean children.**

Method: **Seven *Toll-like receptor* genes (TLR-1, -2, -3, -4, -5, -6, -9) including 15 single-nucleotide polymorphisms were analyzed on 104 Korean children with AITD [Hashimoto′s disease (HD) = 40, Graves′ disease (GD) = 60 (thyroid-associated ophthalmopathy (TAO) = 29, non-TAO = 31)] and 192 healthy individuals.**

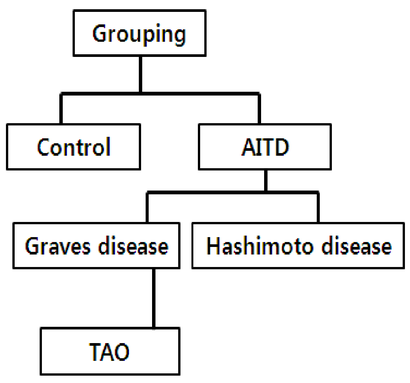

Fig 1. Subjects classifications

Table 1. Characteristics of 104 AITD patients

Characteristics	
Sex (F/M)	86/18
Age (years) at diagnosis	11.3±3.2
Age (years) at enrollment	13.2±3.5
HD condition at diagnosis	
Euthyroid state	9 (20.5%)
Subclinical hypothyroid state	6 (13.6%)
Overt hypothyroid state	23 (52.3%)
Hyperthyroid state	6 (13.6%)
HD patients on T4 replacement	25 (56.8 %)
Class of TAO	
0~1 No sign~ only sign	75
2 soft tissue involvement	7
3 Proptosis	19
4 Extraocular muscle involvement	3
5 Corneal involvement	0
6 Sight loss	0

Table 2. Primer sequences for 15 SNP of 7 TLRs

Gene	SNP		Primer Sequence
TLR1	rs4833095 (+742)	F	GGATCCTAAT GAAAGAATTC CAAGTTGTTT CAATGTTGTT TAAGGTAATA
		R	AAG ACC CTG AGG GCC TTC AAG AC
TLR2	rs4696480 (-16934)	F	AACAGAAATTTATCCATTCATGGTT
		R	AGCAGTTTATTGTGAGAATGAGTTT
	rs1898830 (intron1)	F	CCCATGGGTC AAAAAATAAT CAG
		R	TAT TTT CTA GCA CAT TAA TTT CTA TTC TTA TAT
	rs7656411 (3' UTR)	F	TCT GGT CTT CCT CAG CCT CTA AC
		R	CTA CCT TTA AAT TAC TGT GTA TCA AAC TAT TTT
TLR3	rs3775291 (c.1243)	F	GCC GTG CTA AGT TGT TAT GCT GC-->ATTCCTGGCC TGTGAGTTCT TGC
		R	ACT TTG ACA AAT GAA ACA TTT GTA TCA CTT GCT
	rs3775296 (-7)	F	CCGTTTGATGTATGACTTG
		R	AAGTTGGCGGCTGGTAATCT
TLR4	rs11536889 (3'UTR)	F	GAG ACACAGATGG CTGGGA
		R	TTC TGA GGA GGC TGG ATG AA
	rs10759932 (-1607)	F	TATGATTAAA AGTGATTACC ACATTTTACA GACCAGAAAG TAATAATACG
		R	GAC ACT TGC ATT GTT GCC ACA CG
	rs1927911 (Intron 1)	F	GCAGCAAATC ACCCTGGCAC ACA
		R	AGA TTT CCC CCT ATT TCT ACA TCA CTT TGC TCA
TLR5	rs5744168 (+1174)	F	ACACTC AAGGATTTGA AGGTTCTG
		R	GAT ATC GGG TAT GCT TGG AAT AAA ATG AAT GGT
TLR6	rs5743810 (+745)	F	GCATTTCCAAGTCGTTTCTATGT
		R	GCAAAAACCCTTCACCTTGTT
	rs2381289 (c.*1833)	F	ATA CCC TCT TCC CTT GCA ATG GC
		R	TCC TGA ATC TTG GGC AGA TAC CAT AAA TTT TAG
TLR9	rs352140 (+2848)	F	AAGCTGGACCTCTACCACGA
		R	TTGGCTGTGGATGTTGTT
	rs187084 (-1486)	F	ACTATGGAGCCTGCCTGCCATGATACC
		R	ATCCAGCCTTCTTACAAACCTCCCACCC
	rs352162 (3'UTR)	F	AGATAGTGG TGCGCGGCTT CTCT
		R	GAC TAT TCT GGC CAC AAT CAG G

Table 3. Allele frequencies of TLR polymorphism in controls and AITD

	SNP Alleles	Normal n=183 (%)	AITD n=104(%)
TLR1 (+742)	C	163 (89.1%)	92(88.5%)
rs4833095	T	111 (60.7%)	70(67.3%)
TLR2 (-16934)	A	144 (78.7%)	84(80.8%)
rs4696480	T	140 (76.5%)	73(70.2%)
TLR2 (intron1)	A	142 (77.6%)	83(79.8%)
rs1898830	G	140 (76.5%)	75(72.1%)
TLR2 (3' UTR)	G	129 (70.5%)	82(78.8%)
rs7656411	T	132 (72.1%)	70(67.3%)
TLR3 (c.1243)	A	92 (50.3%)	51(49.0%)
rs3775291	G	164 (89.6%)	96(92.3%)
TLR3 (-7)	A	72 (39.3%)	41(39.4%)
rs3775296	C	175 (95.6%)	96(92.3%)
TLR4 (3'UTR)	C	71 (38.8%)	41(39.4%)
rs11536889	G	171 (93.4%)	96(92.3%)
TLR4 (-1607)	C	86 (47.0%)	41(39.4%)
rs10759932	T	166 (90.7%)	98(94.2%)
TLR4 (Intron 1)	C	156 (85.2%)	90(86.5%)
rs1927911	T	120 (65.6%)	59(56.7%)
CC-TLR5 (+1174)	C	183 (100.0%)	104(100.0 %)
rs5744168	T	4 (2.2%)	3(2.9%)
CC-TLR6 (+745)	C	183(100.0%)	104(100.0%)
rs5743810	G	0(0.0%)	0(0.0%)
TLR6 (c.*1833)	C	139(76.0%)	87(83.7%)
rs2381289	T	142(77.6%)	74(71.2%)
TLR9 (+2848)	A	117 (63.9%)	69(66.3%)
rs352140	G	150 (82.0%)	84(80.8%)
TLR9 (-1486)	C	117 (63.9%)	71(68.3%)
rs187084	T	148 (80.9%)	84(80.8%)
TLR9 (3'UTR)	C	117 (69.9%)	70(67.3%)
rs352162	T	147 (80.3%)	84(80.8%)

Table 4. Significant association of *TLR 3, 4* genes with GD and HD patients

		Normal n=183 (%)	GD n= 60 (%)	HD n= 44 (%)
TLR3 (-7)	AA	8 (4.4%)	2 (3.3%)	6 (13.6%) [d]
rs3775296	AC	64 (35.0%)	20 (33.3%)	13 (29.5%)
	CC	111 (60.7%)	38 (63.3%)	25 (56.8%)
	A	72 (39.3%)	22 (36.7%)	19 (43.2%)
	C	175 (95.6%)	58 (96.7%)	38 (86.4%) [e]
TLR4 (Intron 1)	CC	63 (34.4%)	32 (53.3%) [a]	13 (29.5%)
rs1927911	CT	93 (50.8%)	20 (33.3%) [b]	25 (56.8%)
	TT	27 (14.8%)	8 (13.3%)	6 (13.6%)
	C	156 (85.2%)	52 (86.7%)	38 (86.4%)
	T	120 (65.6%)	28(46.7%) [c]	31 (70.5%)

TLR4 (rs1927911)

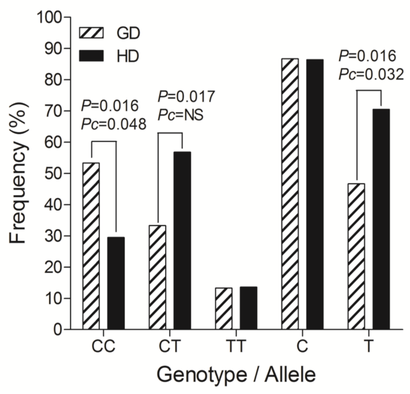

Fig 2. The frequencies of the TLR4 *rs1927911* CC genotype in HD was lower, whereas *TLR4 rs 1927911* CT genotype in HD and *TLR4 rs1927911* T allele in HD showed higher frequencies than GD.

Table 5. Association of *TLR 4,9* genes with TAO and non-TAO patients

		Normal n=183 (%)	Non-TAO n= 31(%)	TAO n= 29(%)
TLR4 (Intron 1)	CC	63 (34.4%)	17 (54.8%) [a]	15 (51.7%)
rs1927911	CT	93 (50.8%)	11 (35.5%)	9 (31.0%)
	TT	27 (14.8%)	3 (9.7%)	5 (17.2%)
	C	156 (85.2%)	28 (90.3%)	24 (82.8%)
	T	120 (65.6%)	14(45.2%) [b]	14 (48.3%)
TLR-9 (-1486)	CC	35 (19.1%)	8 (27.6%)c	2 (6.5%)
rs187084	CT	82 (44.8%)	12 (41.4%)	19 (61.3%)
	TT	66 (36.1%)	9 (31.0%)	10 (32.3%)
	C	117 (63.9%)	20 (69.0%)	21 (67.7%)
	T	148 (80.9%)	21 (72.4%)d	29 (93.5%)

Result-I:

•In total AITD, the frequencies of these alleles had no statistical difference with controls (Table 3).

•In HD, the frequencies of the TLR3 rs3775296 AA genotype (OR=3.45, *P* < 0.022) was higher, whereas the TLR3 rs3775296 C allele (OR=0.29, c*P* < 0.044) showed lower frequencies than in the healthy controls. In GD, the frequencies of the *TLR4 rs1927911* CC genotype (OR=2.18, c*P* < 0.027) was higher, whereas the *TLR4 rs1927911* CT genotype (OR=0.48, *P* < 0.018) and *TLR4 rs1927911* T allele (OR=0.46, c*P* < 0.018) showed lower frequencies than in the healthy controls(Table 4).

Result-II:

• In TAO, the frequencies of the TLR4 *rs1927911* CC genotype was higher, whereas TLR4 *rs1927911* T allele (OR=0.43, *P* < 0.029) showed lower frequencies than in the healthy controls. Between TAO and non-TAO, the frequencies of the TLR9 *rs 187084* CC genotype in non-TAO (OR=5.52, *P* < 0.028) was higher, whereas TLR9 *rs 187084* T allele in non-TAO (OR=0.18, *P* < 0.028) was lower than TAO. However, the statistical significance was disappeared after correction.

Conclusion: **Our results suggest that *TLR*-3 *and -4* gene polymorphisms may contribute to the pathogenesis of HD and GD.**

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