Supplementary data:

Association of *CTLA4, CD28* and *ICOS* gene polymorphisms with clinicopathologic characteristics of childhood IgA nephropathy in Korean population

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Table 1. Primer sequence for the SNPs of *CTLA4*, *CD28* and *ICOS* genes.

Gene	SNP	Function	5′-Sequence-3′	Product size (bp)
CD28 (2q33)	rs1879877	Promoter	Forward: CTTAACAGACCAACAGGTGGAAG	683
	rs3181097	Promoter	Reverse: AAGCCTCCTCCAACTCACTTCTA	
	rs2140148	Intron 1	Forward: AGTAGATTGGCTCTGGAAGCTCT Reverse: TCTGTTAGGAATTGTGCTTGTGA	683
	rs3116494	Intron 2	Forward: TTTCAAATGCAGTCCTGAAAACT Reverse: GAAGGAAGGAAAAAGAAAATGGA	410
CTLA4 (2q33)	rs231777	Intron 1	Forward: TGAATACATTTGAGCTGGGTTTC Reverse: ACTAAATGCGGTCACACTCAACT	710
	rs231779	Intron 1	Forward: CATCTCTGATAGGCAGAGGTGAG Reverse: AGTAGCTGTGTCTTGATGCACTG	700
ICOS (2q33)	rs4270326	Intron 3	Forward: TGAAGTTCTGGTTACCCATAGGA	690
	rs11571314	Intron 3	Reverse: CACAGGGAGATTTTTGTTGTTGT	
	rs10183087	Exon5-UTR	Forward: TCGAGAGCTAAACTTCTCTGGAA Reverse: ATGATAGTGAAATGCGGACAGAT	697
	rs4404254	Exon5-UTR	Forward: TTGTGCCCTCAATTTTCTTTTTA Reverse: GCTCTTCACAGATCCTGGAATTA	691
	rs1559931	Exon5-UTR	Forward: AGCCTGAAAGCTGCAGTTACTAT Reverse: ATGACATATCGGGTCTCACTTGT	701
	rs1365828	3-near gene	Forward: TATGGGAACCATTGCCTGCCTTC Reverse: GGCATGTGAAGTCAGATTGGGTAG	494
	rs768180	3-near gene	Forward: CCCACACGTGTGATTCAATTTGG Reverse: TGGTCAGAGGTTGTCAGTAGAGG	485
	rs6726035	3-near gene	Forward: GGAGACAGCTCAAAAGCTTTCTA Reverse: GAGACAAGAAGCAGGTGTTTCTT	369

Table 2. Logistic regression analysis of *CD28*, *CTLA4* and *ICOS* polymorphisms in IgAN patients subgrouped according to the presence of nephrotic range proteinuria (>40 mg/m^2/h) after adjustment for gender and age.

Gene symbol	SNPs	Genotype	≤40 mg/m^2/h *n* (frequency)	>40 mg/m^2/h *n* (frequency)	Model	OR (95% CI)	*P* value
CD28	rs1879877	A/A	66 (43.1%)	4 (22.2%)	Codominant	**2.15 (1.06–4.36)**	**0.032**
	Promoter	A/C	69 (45.1%)	9 (50%)	Dominant	2.67 (0.84–8.48)	0.076
		C/C	18 (11.8%)	5 (27.8%)	Recessive	2.91 (0.92–9.13)	0.085
	rs3181097	A/A	48 (31.6%)	3 (16.7%)	Codominant	1.79 (0.88–3.64)	0.100
	Promoter	A/G	73 (48%)	9 (50%)	Dominant	2.35 (0.65–8.52)	0.160
		G/G	31 (20.4%)	6 (33.3%)	Recessive	2.06 (0.71–6.01)	0.200
	rs2140148	T/T	124 (81%)	16 (88.9%)	Codominant	0.55 (0.12–2.47)	0.400
	Intron 1	T/G	28 (18.3%)	2 (11.1%)	Dominant	0.55 (0.12–2.56)	0.420
		G/G	1 (0.6%)	0 (0%)	Recessive	NA (0.00–NA)	0.670
	rs3116494	A/A	64 (88.9%)	84 (84%)		0.35 (0.05–2.70)	0.317**
	Intron 2	A/G	8 (11.1%)	16 (16%)			
CTLA4	rs231777	C/C	115 (75.7%)	11 (64.7%)	Codominant	2.38 (0.95–6.00)	0.075
	Intron 1	T/C	37 (24.3%)	4 (23.5%)	Dominant	1.70 (0.59–4.92)	0.340
		T/T	0 (0%)	2 (11.8%)	Recessive	NA (0.00–NA)	0.002
	rs231779	T/T	74 (48.4%)	4 (23.5%)	Codominant	**2.85 (1.28–6.33)**	**0.009**
	Intron 1	T/C	70 (45.8%)	9 (52.9%)	Dominant	3.08 (0.96–9.89)	0.043
		C/C	9 (5.9%)	4 (23.5%)	Recessive	**4.96 (1.33–18.52)**	**0.028**
ICOS	rs4270326	C/C	119 (77.3%)	14 (77.8%)	Codominant	0.86 (0.29–2.55)	0.790
	Intron 3	G/C	32 (20.8%)	4 (22.2%)	Dominant	0.94 (0.29–3.07)	0.920
		G/G	3 (2%)	0 (0%)	Recessive	0.00 (0.00–NA)	0.390
	rs11571314	A/A	113 (73.4%)	14 (77.8%)	Codominant	0.73 (0.25–2.13)	0.550
	Intron 3	A/G	37 (24%)	4 (22.2%)	Dominant	0.78 (0.24–2.53)	0.680
		G/G	4 (2.6%)	0 (0%)	Recessive	0.00 (0.00–NA)	0.320
	rs10183087	A/A	113 (73.4%)	14 (77.8%)	Codominant	0.73 (0.25–2.13)	0.550
	Exon 5-UTR	A/C	37 (24%)	4 (22.2%)	Dominant	0.78 (0.24–2.53)	0.680
		C/C	4 (2.6%)	0 (0%)	Recessive	0.00 (0.00–NA)	0.320
	rs4404254	T/T	113 (73.9%)	14 (82.3%)	Codominant	0.58 (0.17–1.95)	0.340
	Exon 5-UTR	T/C	36 (23.5%)	3 (17.6%)	Dominant	0.60 (0.16–2.20)	0.420
		C/C	4 (2.6%)	0 (0%)	Recessive	0.00 (0.00–NA)	0.340
	rs1559931	G/G	113 (73.4%)	14 (77.8%)	Codominant	0.73 (0.25–2.13)	0.550
	Exon 5-UTR	A/G	37 (24%)	4 (22.2%)	Dominant	0.78 (0.24–2.53)	0.680
		A/A	4 (2.6%)	0 (0%)	Recessive	0.00 (0.00–NA)	0.320
	rs1365828	C/C	69 (44.8%)	5 (27.8%)	Codominant	1.82 (0.91–3.67)	0.093
	3-near gene	A/C	68 (44.2%)	9 (50%)	Dominant	2.27 (0.74–6.89)	0.130
		A/A	17 (11%)	4 (22.2%)	Recessive	2.27 (0.67–7.73)	0.210
	rs768180	C/C	48 (31.6%)	6 (33.3%)	Codominant	0.96 (0.47–1.98)	0.910
	3-near gene	T/C	79 (52%)	9 (50%)	Dominant	0.92 (0.32–2.60)	0.870
		T/T	25 (16.4%)	3 (16.7%)	Recessive	1.00 (0.27–3.73)	1.000
	rs6726035	T/T	39 (25.5%)	7 (38.9%)	Codominant	0.95 (0.48–1.89)	0.880
	3-near gene	T/C	79 (51.6%)	5 (27.8%)	Dominant	0.54 (0.19–1.48)	0.240
		C/C	35 (22.9%)	6 (33.3%)	Recessive	1.73 (0.60–4.97)	0.320

The statistically significant single nucleotide polymorphisms (SNPs) are shown, of a total of 14 SNPs genotyped from *CD28*, *CTLA4* and *ICOS* in 172 patients with IgAN. Genotype distributions are shown as numbers (%), odds ratios (OR), 95% confidence intervals (CI). *P* values were obtained by logistic regression analysis using codominant, dominant and recessive models after controlling for gender and age. Total numbers of SNPs differ, because the genotypes of some SNPs were unreadable. *The allele frequencies of *CD28* rs3116494 did not have all three genotypes present in the study population, only allele frequencies were compared by the chi-square test.

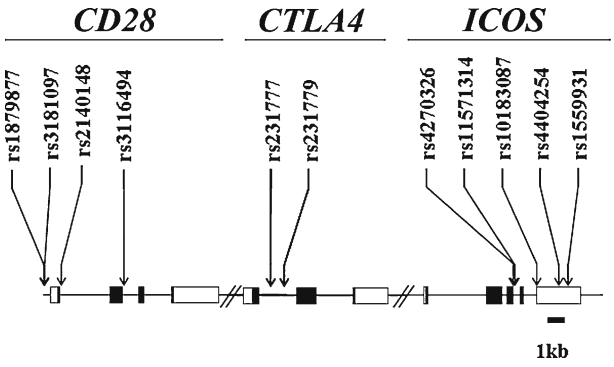

Figure 1. Location of genetic polymorphisms in the chromosomal region 2q33 harbouring *CD28*, *CTLA4* and *ICOS* genes. Distances are not to scale. The three SNPs of *ICOS* were not indicated. The locations of SNPs are too far from 3'UTR. The SNPs are locataed about 11∼290 kbp from end of 3'UTR of *ICOS*.

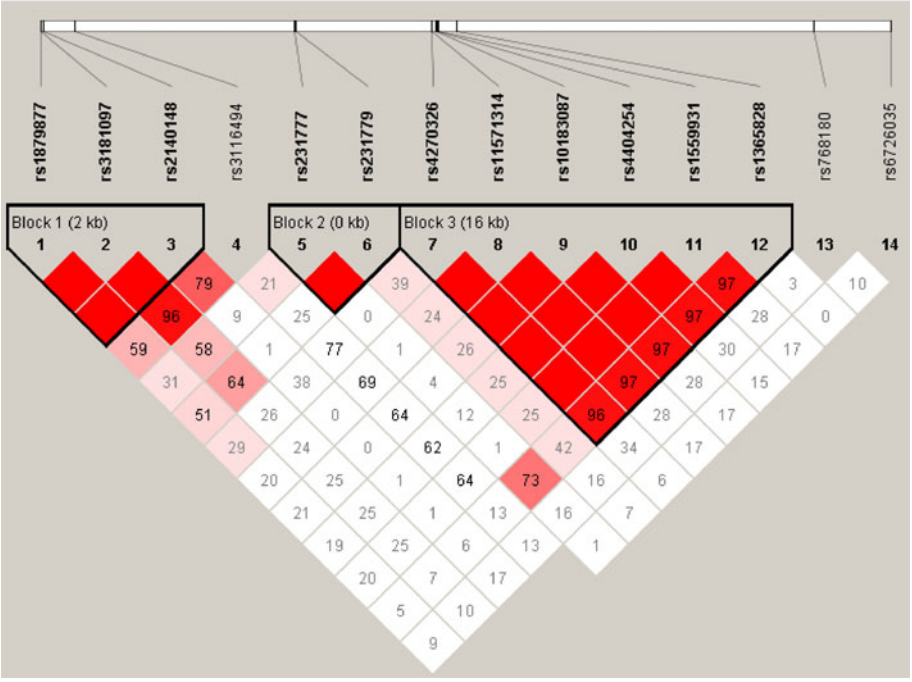

Figure 2. Linkage disequilibrium among polyorphisms of *CD28*, *CTLA4* and *ICOS* genes. Each box represents linkage disequilibrium (LD) (range 0–1) between pairs of single-nucleotide polymorphism (SNP) markers as generated by Haploview. Red shading indicates strong LD (no number entry means a score 1). Purple shading indicates 'uninformative', and white shading indicates strong evidence of recombination.