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Linkage and Association Study of Neurotrophins and Their Receptors as Novel Susceptibility Genes for Childhood IgA Nephropathy

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ABSTRACT: Neurotrophins (NTs) and their receptors (NTRs) are known to be important for pathogenesis of various inflammatory diseases that occur in not only neuronal but also nonneuronal tissues, including kidney. Here, we investigated association between childhood IgA nephropathy (IgAN) and single nucleotide polymorphisms (SNPs) of genes encoding NTs [nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF)] and NTRs [nerve growth factor receptor (NGFR) and neurotrophic tyrosine kinase receptor 1–3 (NTRK1–3)]. The genotyping data of 197 patients and 289 control subjects revealed significant association between NGF SNP rs11102930 and presence of IgAN. Patient subgroup analysis revealed that that the presence of nephrotic range proteinuria (>40 mg/m^2/h) was associated with rs6334 of NTRK1 and rs11030104, rs7103411, rs7103873, and rs6484320 of BDNF. Significant genotype differences were observed in podocyte foot process effacement for rs1187321 and rs1187323 of NTRK2. Furthermore, some SNPs showed significantly different genotype distribution between patients with or without pathologically advanced disease markers, specifically in rs6334 of NTRK1. Our results suggest that SNPs of NTs and NTRs are associated with susceptibility, pathological advancement, podocyte foot process effacement, and development of proteinuria in childhood IgAN. **(*Pediatr Res* 69: 299–305, 2011)**

IgA nephropathy (IgAN) is the most commonly occurring form of chronic glomerulonephritis (GN) in pediatric patients and is defined by IgA deposition in the glomerular mesangium accompanied by a mesangial proliferative GN. The extent and intensity of glomerular injury in response to mesangial IgA deposition are extremely variable and determine the levels of subsequent mesangial cell proliferation and interstitial fibrosis. Polymorphisms of candidate genes that are involved in these processes could thus affect disease susceptibility and progression.

The neurotrophins (NTs) are a family of neurotrophic factors that are essential for the development of the nervous system, regulating the survival, death, tissue repair, and differentiation of neurons (1). There are four NT family members: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) (1). The biological effects of all NTs are mediated by neurotrophin receptors (NTRs), which include tyrosine kinase receptors (TrkA, B, and C) and nerve growth factor receptor (NGFR, also known as NT receptor p75). NTs bind to a common family of Trk cell surface receptors with high affinity and variable specificity. NGF binds preferentially to TrkA (encoded by *NTRK1*), BDNF and NT-4/5 bind to TrkB (encoded by *NTRK2*), and NT-3 binds to TrkC (encoded by *NTRK3*). All NTs also bind to the common low-affinity NT receptor NGFR (encoded by *NGFR*) (1). Signaling through Trk receptors includes different pathways, such as the Ras/ERK pathway, the phosphatidylinositol-3-kinase (PI3K)/Akt kinase pathway, the phospholipase Cγ (PLCγ) pathway, and the Smad transduction pathway (1,2). The NGFR-mediated signaling is more complex, involving both Trk-dependent and -independent signal transductions.

NTs have been studied mainly in relation to neurological and psychological diseases. However, increasing evidences indicate that NTs and NTRs are implicated in the pathogenesis of various inflammatory diseases (3). Moreover, NTs were found to be expressed in the kidney tissues, and it has been suggested that the NTs has an enough possibility to be involved in the pathophysiology of renal disease, including IgAN.

There have been several single nucleotide polymorphisms (SNPs) studies on neuronal and nonneuronal tissue-associated diseases (4–7). However, there have been no studies linking renal diseases with SNPs of genes encoding NT family members and their receptors, especially in pediatric IgAN. Here, we have investigated associations between polymorphisms of NT pathway genes and childhood IgAN.

METHODS

Patients and controls. We examined a total of 197 Korean pediatric patients with IgAN confirmed by biopsy (mean age ± SD, 12.63 ± 5.19 y; 117 boys, 13.39 ± 4.98 y; 80 girls, 11.53 ± 5.31 y) and compared them with 289 healthy control subjects (mean age ± SD, 37.63 ± 13.35 y; 157 males, 39.63 ± 14.72 y; 132 females, 35.25 ± 11.11 y). The follow-up duration before the renal biopsy was 21.7 ± 27.8 mo. Patients were detected through abnormal urinalysis results during school screenings, and most of them showed no other symptoms of GN. Thus, they were assumed to have

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Abbreviations: BDNF, brain-derived neurotrophic factor; **GN,** glomerulonephritis; **IgAN,** IgA nephropathy; **LD,** linkage disequilibrium; **NGF (R),** nerve growth factor (receptor); **NT (R),** neurotrophin (receptor); **NTF-3,** neurotrophin-3; **NTRK,** neurotrophic tyrosine kinase receptor; **SNP,** single nucleotide polymorphism; **Trk,** tyrosine kinase receptor

relatively early stage disease. At our center, we performed a renal biopsy in all patients with i) unexplained prolonged isolated hematuria or proteinuria of duration ≥ 12 mo, ii) concomitant hematuria and proteinuria for > 3 mo, iii) a second episode of gross hematuria with decreased serum C3 and C4 levels, or iv) decreased renal function. Healthy control subjects were also recruited based on routine screenings. This screening included the completion of a questionnaire addressing the presence of symptoms, medical history, measurement of blood pressure, electrocardiography, abdominal sonography, and laboratory tests such as complete blood count, fasting glucose level, total cholesterol, triglyceride, HDL-cholesterol, rheumatoid factor, hepatitis viral markers, Hb A1C, liver enzymes, blood urea nitrogen, creatinine, electrolytes, and urinalysis findings (protein, glucose, and occult blood). Control candidates with an abnormal result for any item were excluded.

Patient subgroups. To determine the natures of the associations between SNPs of NTs and NTRs and the development of proteinuria, we divided patients into subgroups according to the largest amount of proteinuria observed during the course of disease (nephrotic range proteinuria >40 and ≤40 mg/m^2/h).

In addition, patients were allocated to gross hematuria (+) and (−) groups according to the presence of gross hematuria episodes as an initial symptom of IgAN. Patients with IgAN were also divided into mild and advanced disease subgroups to evaluate the contribution of the SNPs in disease progression in terms of pathologic findings. Members of the advanced disease group had at least one of the following pathological markers: interstitial fibrosis, tubular atrophy, or global sclerosis. Finally, we further divided patients with IgAN into podocyte foot process effacement (+) and (−) subgroups according to renal biopsy. The demographic characteristics of patients with IgAN were summarized, with small differences in subgroup numbers resulting from the loss of some clinical data (Table 1).

This study was approved by the ethics review committee of the Medical Research Institute, Kyung Hee University Medical Center, Seoul. Written informed consent was obtained from all subjects and from the parents or legal guardians.

SNP selection and genotyping. Three SNPs of *NGF*, one SNP on *NGFR*, four SNPs of *BDNF*, four SNPs of *NTRK1*, two SNPs of *NTRK2*, and one SNP of *NTRK3* were selected based on the findings of extensive database searches (http://www.ebi.ac.uk/ensemble/ and http://ncbi.nlm.nih.gov/SNP) for heterozygosity >0.1 and a minor allele frequency (MAF) of >0.05 (Table 2).

DNA was isolated from peripheral blood samples using Core One Blood Genomic DNA Isolation Kits (CoreBioSystem, Seoul). SNP genotyping was conducted by direct sequencing. Genomic DNA was amplified using specific primers for the 15 SNPs of NTs and their receptor genes. The samples were sequenced using an ABI Prism 377 automatic sequencer (PE Applied Biosystems, Foster City, CA), and the sequence data were analyzed using SeqManII software (DNASTAR Inc., Madison, WI).

Statistical analysis. We analyzed 15 SNPs of genes encoding NTs and their receptors in the 197 patients and 289 controls. For the case-control association study, Hardy-Weinberg equilibrium (HWE) for all SNPs was assessed using SNPstats (Biostatistics and Bioinformatics Unit, Barcelona, Spain) in both cases and controls (8), and the SNPs not in HWE ($p < 0.05$) were excluded from the analysis. For the logistic regression analysis and trend test, we used SNPstats and SNPAnalyzer (ISTECH Inc., Goyang, Korea). To show alternative effects of the variants, logistic regression analysis was performed in the statistical genetic models (codominant, dominant, recessive, and overdominant models) (9). For the SNPs that do not have all three genotypes present in the study population, only allele frequencies were compared by the χ^2 test. And they were taken into account of Bonferroni's correction. To reduce experimental error, we calculated sample power for the SNPs and the number of cases was adjusted to achieve 80% power ($\alpha = 0.05$, genotype relative risk = 2-fold) using a genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc).

A linkage disequilibrium (LD) block of polymorphisms and haplotype analysis was tested using Haploview version 4.2 (Broad Institute, Cambridge, MA), HapAnalyzer version 1.0 (http://hap.ngri.go.kr/), and HelixTree (Golden Helix Inc., Bozeman, MT). We examined Lewontin's D' and r^2 between all pairs of biallelic loci (10). The haplotypes and their frequencies were inferred using the expectation-maximization (EM) algorithm (11).

RESULTS

Despite the fact that most patients were asymptomatic at presentation, analysis of the subgroups of patients with IgAN showed that the presence of podocyte effacement were correlated with the advanced pathologic classification of IgAN according to the H. S. Lee's glomerular grading system (12) (Table 1).

The genotypic distributions of all SNPs in this study were consistent with Hardy-Weinberg equilibrium ($p > 0.05$). The genotyping data of 197 patients and 289 controls revealed a significant association between *NGF* rs11102930 and the presence of IgAN by logistic regression analysis after adjusting for gender (codominant model: OR = 0.67, 95% CI = 0.51–0.88, $p = 0.003$; dominant model: OR = 0.62, 95% CI = 0.42–0.91, $p = 0.015$; recessive model: OR = 0.56, 95% CI = 0.34–0.90, $p = 0.015$; Table 3). We calculated sample power for this SNP, rs11102930. In our case-control study, we had 0.97 for codominant model [effective sample size (ESS) = 239], 0.98 for dominant model (ESS = 152), 0.53 for recessive model (ESS = 163) power to detect a 2-fold increased risk, assuming an α-level of 0.05. Thus, codominant and

Table 1. *Demographics of the patients with IgA nephropathy (n = 197)*

Subgroup	*n* (%)	M:F	Age (y; mean ± SD)	Follow-up duration before renal biopsy (months) Mean	(months)	Pathologic Grading (*n*)* I	II	III	IV	V	*p*
Proteinuria (mg/m^2/h)†											
>40	33 (16.8)	23:10	11.41 ± 4.76	104.48 ± 78.77	21.7 ± 27.7	17	16	0	0	0	0.072
≤40	164 (83.2)	94:70	12.84 ± 5.22	10.71 ± 9.68	21.8 ± 27.8	115	48	0	1	0	
Gross hematuria‡											
+	37 (18.8)	21:16	12.88 ± 7.68	NA§	21.9 ± 28.1	24	12	0	1	0	0.384
−	160 (81.2)	96:64	12.53 ± 4.41	NA	21.7 ± 27.8	108	52	0	0	0	
Advanced disease markers											
+	21 (10.7)	16:05	15.71 ± 6.72	NA	21.5 ± 27.6	6	14	0	1	0	<0.001
−	176 (89.3)	101:75	12.22 ± 4.83	NA	21.7 ± 27.8	126	50	0	0	0	
Podocyte foot process effacement											
+	72 (36.5)	44:28	12.80 ± 5.34	NA	21.6 ± 27.7	33	38	0	1	0	<0.001
−	125 (63.5)	73:52	12.48 ± 5.07	NA	21.6 ± 27.8	99	26	0	0	0	

*Modified H. S. Lee's glomerular grading system (12); Chi-square test was performed to determine the correlation between pathologic grading and the presence of proteinuria, gross hematuria, advanced disease markers, and podocyte foot process effacement.

†Proteinuria indicates the largest level of proteinuria observed during the course of disease.

‡Gross hematuria developed as a first symptom of IgA nephropathy.

§NA, not applicable.

Table 2. *Information and sequences of primers of candidate SNPs of neurotrophins and their receptor-encoding genes*

Gene	SNPs	Function	Primers and conditions			
				Sequence (5′–3′)	Product size (bp)	Annealing temperature (°C)
NGF (1p13)	rs6330	Ala273Val	Forward	CATTCCAGGTGCATAGCGTAAT	328	65
			Reverse	AGATCCTGAGTGTCTGCAGCTT		
	rs3738701	Intron	Forward	GGAGAGGTGAGGGAAGCTG	321	65
			Reverse	CTCCCGTACTCCTGAGTCACAC		
	rs11102930	Promoter	Forward	AACAGTTTTACCAAGGGAGCAG	355	62
			Reverse	GAGTTGTGTGGAGGGTCTGACT		
NTRK1 (1p21)	rs926103	Promoter	Forward	CTTCATCTATTCCTGGCAGGTG	381	59
			Reverse	TAGACAGGAGAGGCTGGGTATG		
	rs2150906	Promoter	Forward	TCCAGAGACTCTCCTTCCTTTG	312	62
			Reverse	GCACTCTGTGTCAGTGACCATT		
	rs1800601	5′UTR	Forward	AAATTATTGACTGGGCAGGAGA	301	62
			Reverse	AAGGACTTGCAGATGGACAAAG		
	rs6334	Synonymous	Forward	CTCCATCACATCAGGACAGAGT	382	62
			Reverse	TGTCTATAGGGAAGGGAAGACG		
NTRK2 (9q22)	rs1187321	Promoter	Forward	ACCACTCGATGTGTGTTACAGC	304	58
			Reverse	AGAGAGCAATGGGTTGGAGTCT		
	rs1187323	Promoter	Forward	GCGCATCTGGCGCCAGAGCGCG	425	65
			Reverse	GTGTTCATGTGTGCTAGGGTGT		
BDNF (11p13)	rs11030104	Intron	Forward	AGAGTCATCCGAAGGTTGAAAA	353	58
			Reverse	TGCGGATCCCTGCTCTAAGGAA		
	rs7103411	Intron	Forward	TGCATCTGATTTCAGAGGTGAG	387	62
			Reverse	AAACATAGGAGGGGAAAAGGAC		
	rs7103873	Intron	Forward	GGGAAGTCTTGAATTTTTGTGC	377	62
			Reverse	GGGAGCGCACTGTAAAGATACT		
	rs6484320	Intron	Forward	GCAGTGCTTGGCATAGTAAATG	334	59
			Reverse	AGGAACTCAGATAGGGCAGGTT		
NTRK3 (15q25)	rs1128994	Synonymous	Forward	TCTTCGGTTCAGAGGTTCCCTT	418	65
			Reverse	CCTGAAACCAGTCTTCCTATGG		
NGFR (17q21)	rs11466155	Synonymous	Forward	GTTGGATTACACGGTCCACACC	376	59
			Reverse	GCAGATGATGAGTGAGGATGAG		

dominant model of rs11102930 were sufficiently powerful to determine positive association.

When we assessed genetic association between the 15 SNPs and subgroups of patients with IgAN, *NGF* rs6330 was found to be associated with the presence of gross hematuria as an initial IgAN symptom (dominant model: OR = 2.25, 95% CI = 1.06–4.80, $p = 0.036$; overdominant model: OR = 2.27, 95% CI = 1.06–4.88, $p = 0.036$). In addition, the presence of nephrotic range proteinuria was found to be associated with *NTRK1* rs6334 (overdominant model: OR = 2.40, 95% CI = 1.09–5.28, $p = 0.026$), *BDNF* rs11030104 (codominant model: OR = 0.49, 95% CI = 0.28–0.86, $p = 0.010$; recessive model: OR = 0.14, 95% CI = 0.03–0.63, $p = 0.001$), *BDNF* rs7103411 (codominant model: OR = 0.49, 95% CI = 0.28–0.86, $p = 0.010$; recessive model: OR = 0.14, 95% CI = 0.03–0.63, $p = 0.001$), *BDNF* rs7103873 (dominant model: OR = 3.56, 95% CI = 1.17–10.79, $p = 0.012$), and *BDNF* rs6484320 (codominant model: OR = 0.49, 95% CI = 0.28–0.86, $p = 0.010$; recessive model: OR = 0.14, 95% CI = 0.03–0.63, $p = 0.001$; Table 4).

In terms of podocyte foot process effacement, *NTRK2* rs1187321 (recessive model: OR = 5.82, 95% CI = 1.13–29.89, $p = 0.021$) and *NTRK2* rs1187323 (recessive model: OR = 5.82, 95% CI = 1.13–29.89, $p = 0.021$) showed significant differences in genotype distribution (data not shown).

Comparison of genotype differences between the advanced pathologic marker (+) and (−) groups revealed a significant association with *NTRK1* rs6334 (overdominant model: OR = 3.19, 95% CI = 1.16–8.75, $p = 0.018$; data not shown).

In measurements of pair-wise LD, one LD block was identified in the SNPs of *NTRK1* (composed with rs926103, rs2150906, and rs1800601) by the Gabriel method (13), but it was not statistically significant (data not shown).

DISCUSSION

NTs have been studied mainly in relation to neurological and psychological diseases (1). However, there is increasing evidence that NTs and NTRs are also expressed in nonneuronal tissues (1,3), and several recent works have elucidated a regulatory effect of NTs on the proinflammatory mediators, such as IL-1β, TNF-α, and IL-6 (3,14). In addition, BDNF and NGF are detectable and over-expressed in the synovial tissues and fluids of patients with rheumatoid arthritis (RA) and spondyloarthritis but not in healthy controls (15–17). Additional evidence exists for an association between increased levels of NTs and susceptibility to inflammatory diseases such as systemic lupus erythematosus (SLE) (18,19), RA, multiple sclerosis, and juvenile chronic arthritis (20–22). Even a Disease Activity Index in patients with SLE was significantly correlated with serum levels of NGF (19).

In the kidney, the NTs and their receptors are expressed in both fetal and adult renal tissues; renal tubular, interstitial, and glomerular cells, and especially mesangial cells and podocytes (23–28). In addition, NT systems are critical for kidney de-

Table 3. *Logistic regression analysis of neurotrophins and their receptor-encoding gene polymorphisms in IgA nephropathy patients and healthy controls after adjustment for age**

Gene symbol	SNP	Genotype	Controls (n = 289; %)	IgAN (n = 197; %)	Model	OR (95% CI)	p
NGF	rs6330	C/C	182 (63)	130 (66.7)	Codominant	0.92 (0.65–1.31)	0.640
	Ala273Val	T/C	104 (36)	60 (30.8)	Dominant	0.85 (0.58–1.25)	0.400
		T/T	3 (1)	5 (2.6)	Recessive	2.42 (0.57–10.27)	0.220
					Overdominant	0.79 (0.54–1.17)	0.240
	rs3738701	C/C	241 (83.7)	173 (87.8)	Codominant	0.73 (0.44–1.22)	0.230
	intron	A/C	46 (16)	23 (11.7)	Dominant	0.70 (0.41–1.19)	0.190
		A/A	1 (0.4)	1 (0.5)	Recessive	1.66 (0.10–26.89)	0.720
					Overdominant	0.68 (0.40–1.17)	0.160
	rs11102930	C/C	76 (26.3)	71 (36.6)	Codominant	0.67 (0.51–0.88)	0.003
	Promoter	T/C	146 (50.5)	95 (49)	Dominant	0.62 (0.42–0.91)	0.015
		T/T	67 (23.2)	28 (14.4)	Recessive	0.56 (0.34–0.90)	0.015
					Overdominant	0.94 (0.65–1.35)	0.740
NTRK1	rs926103	G/G	198 (68.8)	137 (69.5)	Codominant	0.95 (0.67–1.34)	0.770
	Promoter	A/G	82 (28.5)	55 (27.9)	Dominant	0.95 (0.64–1.41)	0.790
		A/A	8 (2.8)	5 (2.5)	Recessive	0.90 (0.29–2.80)	0.860
					Overdominant	0.96 (0.64–1.44)	0.830
	rs2150906	G/G	250 (87.4)	174 (88.8)	Codominant	0.84 (0.48–1.46)	0.530
	Promoter	A/G	35 (12.2)	22 (11.2)	Dominant	0.86 (0.49–1.51)	0.600
		A/A	1 (0.4)	0 (0)	Recessive	0.00 (0.00-NA)	0.290
					Overdominant	0.89 (0.50–1.57)	0.680
	rs1800601	T/T	198 (68.8)	137 (69.5)	Codominant	0.95 (0.67–1.34)	0.770
	5′UTR	T/C	82 (28.5)	55 (27.9)	Dominant	0.95 (0.64–1.40)	0.780
		C/C	8 (2.8)	5 (2.5)	Recessive	0.90 (0.29–2.80)	0.860
					Overdominant	0.96 (0.64–1.43)	0.830
	rs6334	G/G	111 (38.7)	81 (41.1)	Codominant	0.94 (0.72–1.24)	0.660
	Synonymous	A/G	141 (49.1)	92 (46.7)	Dominant	0.89 (0.61–1.29)	0.540
		A/A	35 (12.2)	24 (12.2)	Recessive	1.01 (0.58–1.76)	0.980
					Overdominant	0.89 (0.62–1.28)	0.530
NTRK2	rs1187321	A/A	164 (56.8)	118 (59.9)	Codominant	0.87 (0.64–1.19)	0.390
	Promoter	A/T	109 (37.7)	71 (36)	Dominant	0.88 (0.61–1.26)	0.480
		T/T	16 (5.5)	8 (4.1)	Recessive	0.73 (0.30–1.73)	0.460
					Overdominant	0.93 (0.64–1.35)	0.690
	rs1187323	A/A	165 (57.1)	118 (59.9)	Codominant	0.88 (0.65–1.20)	0.430
	Promoter	A/C	108 (37.4)	71 (36)	Dominant	0.89 (0.61–1.28)	0.530
		C/C	16 (5.5)	8 (4.1)	Recessive	0.73 (0.31–1.75)	0.480
					Overdominant	0.94 (0.64–1.37)	0.740
BDNF	rs11030104	A/A	77 (26.6)	51 (25.9)	Codominant	1.12 (0.86–1.46)	0.380
	Intron	A/G	155 (53.6)	97 (49.2)	Dominant	1.03 (0.68–1.56)	0.880
		G/G	57 (19.7)	49 (24.9)	Recessive	1.33 (0.86–2.06)	0.200
					Overdominant	0.84 (0.59–1.21)	0.350
	rs7103411	T/T	79 (27.3)	51 (25.9)	Codominant	1.14 (0.88–1.48)	0.330
	Intron	T/C	153 (52.9)	97 (49.2)	Dominant	1.07 (0.71–1.61)	0.750
		C/C	57 (19.7)	49 (24.9)	Recessive	1.33 (0.86–2.06)	0.200
					Overdominant	0.86 (0.60–1.24)	0.430
	rs7103873	G/G	75 (25.9)	56 (28.4)	Codominant	0.96 (0.74–1.25)	0.770
	Intron	G/C	155 (53.6)	100 (50.8)	Dominant	0.89 (0.59–1.33)	0.570
		C/C	59 (20.4)	41 (20.8)	Recessive	1.03 (0.66–1.61)	0.900
					Overdominant	0.89 (0.62–1.28)	0.540
	rs6484320	A/A	80 (27.7)	51 (25.9)	Codominant	1.15 (0.88–1.49)	0.310
	Intron	T/A	152 (52.6)	97 (49.2)	Dominant	1.09 (0.72–1.64)	0.690
		T/T	57 (19.7)	49 (24.9)	Recessive	1.33 (0.86–2.06)	0.200
					Overdominant	0.88 (0.61–1.26)	0.470
NTRK3	rs1128994	C/C	185 (64.5)	124 (63.6)	Codominant	1.08 (0.77–1.51)	0.650
	Synonymous	T/C	96 (33.5)	64 (32.8)	Dominant	1.04 (0.71–1.52)	0.830
		T/T	6 (2.1)	7 (3.6)	Recessive	1.65 (0.54–5.02)	0.380
					Overdominant	0.98 (0.67–1.45)	0.930
NGFR	rs11466155	C/C	251 (86.8)	178 (90.4)	Codominant	0.73 (0.42–1.28)	0.260
	Synonymous	T/C	37 (12.8)	18 (9.1)	Dominant	0.69 (0.39–1.24)	0.210
		T/T	1 (0.4)	1 (0.5)	Recessive	1.66 (0.10–26.89)	0.720
					Overdominant	0.67 (0.37–1.22)	0.180

*Total numbers of SNPs differ because the genotypes of some SNPs were not determined.

Table 4. *Logistic regression analysis of neurotrophins and their receptor-encoding gene polymorphisms in IgA nephropathy patients with and without nephrotic range proteinuria (>40 mg/m²/h) after adjustment for gender and age**

Gene symbol	SNP	Genotype	Proteinuria ≤40 mg/m²/h n = 164 (%)	Proteinuria >40 mg/m²/h n = 33 (%)	Model	OR (95% CI)	p
NGF	rs6330	C/C	109 (66.9)	21 (65.6)	Codominant	1.02 (0.49–2.08)	0.970
	Ala273Val	T/C	50 (30.7)	10 (31.2)	Dominant	0.99 (0.44–2.22)	0.970
		T/T	4 (2.5)	1 (3.1)	Recessive	1.32 (0.14–12.56)	0.810
					Overdominant	0.95 (0.42–2.18)	0.910
	rs3738701	C/C	141 (86)	32 (97)	Codominant	0.16 (0.02–1.27)	0.024
	Intron	A/C	22 (13.4)	1 (3)	Dominant	0.16 (0.02–1.25)	0.025
		A/A	1 (0.6)	0 (0)	Recessive	0.00 (0.00-NA)	0.470
					Overdominant	0.17 (0.02–1.35)	0.034
	rs11102930	C/C	58 (35.8)	13 (40.6)	Codominant	0.73 (0.41–1.30)	0.280
	Promoter	T/C	78 (48.1)	17 (53.1)	Dominant	0.86 (0.39–1.89)	0.700
		T/T	26 (16.1)	2 (6.2)	Recessive	0.32 (0.07–1.46)	0.095
					Overdominant	1.33 (0.61–2.89)	0.470
NTRK1	rs926103	G/G	116 (70.7)	21 (63.6)	Codominant	0.98 (0.47–2.06)	0.970
	Promoter	A/G	43 (26.2)	12 (36.4)	Dominant	1.13 (0.50–2.56)	0.770
		A/A	5 (3)	0 (0)	Recessive	0.00 (0.00-NA)	0.170
					Overdominant	1.31 (0.58–2.98)	0.520
	rs2150906	G	311 (94.8)	59 (91.2)		0.65 (0.23–1.82)	1.000†
	Promoter	A	17 (5.2)	5 (8.8)			
	rs1800601	T/T	116 (70.7)	21 (63.6)	Codominant	0.98 (0.47–2.06)	0.970
	5′UTR	T/C	43 (26.2)	12 (36.4)	Dominant	1.13 (0.50–2.56)	0.770
		C/C	5 (3)	0 (0)	Recessive	0.00 (0.00-NA)	0.170
					Overdominant	1.31 (0.58–2.98)	0.520
	rs6334	G/G	71 (43.3)	10 (30.3)	Codominant	1.22 (0.69–2.15)	0.500
	Synonymous	A/G	71 (43.3)	21 (63.6)	Dominant	1.92 (0.84–4.38)	0.110
		A/A	22 (13.4)	2 (6.1)	Recessive	0.45 (0.10–2.06)	0.260
					Overdominant	2.40 (1.09–5.28)	0.026
NTRK2	rs1187321	A/A	100 (61)	18 (54.5)	Codominant	1.07 (0.56–2.04)	0.850
	Promoter	A/T	57 (34.8)	14 (42.4)	Dominant	1.18 (0.55–2.56)	0.670
		T/T	7 (4.3)	1 (3)	Recessive	0.59 (0.07–5.11)	0.620
					Overdominant	1.29 (0.59–2.82)	0.520
	rs1187323	A/A	100 (61)	18 (54.5)	Codominant	1.07 (0.56–2.04)	0.850
	Promoter	A/C	57 (34.8)	14 (42.4)	Dominant	1.18 (0.55–2.56)	0.670
		C/C	7 (4.3)	1 (3)	Recessive	0.59 (0.07–5.11)	0.620
					Overdominant	1.29 (0.59–2.82)	0.520
BDNF	rs11030104	A/A	39 (23.8)	12 (36.4)	Codominant	0.49 (0.28–0.86)	0.010
	Intron	A/G	78 (47.6)	19 (57.6)	Dominant	0.58 (0.26–1.31)	0.200
		G/G	47 (28.7)	2 (6.1)	Recessive	0.14 (0.03–0.63)	0.001
					Overdominant	1.70 (0.78–3.68)	0.180
	rs7103411	T/T	39 (23.8)	12 (36.4)	Codominant	0.49 (0.28–0.86)	0.010
	Intron	T/C	78 (47.6)	19 (57.6)	Dominant	0.58 (0.26–1.31)	0.200
		C/C	47 (28.7)	2 (6.1)	Recessive	0.14 (0.03–0.63)	0.001
					Overdominant	1.70 (0.78–3.68)	0.180
	rs7103873	G/G	52 (31.7)	4 (12.1)	Codominant	1.72 (0.99–2.99)	0.050
	Intron	G/C	80 (48.8)	20 (60.6)	Dominant	3.56 (1.17–10.79)	0.012
		C/C	32 (19.5)	9 (27.3)	Recessive	1.41 (0.59–3.39)	0.440
					Overdominant	1.80 (0.82–3.92)	0.140
	rs6484320	A/A	39 (23.8)	12 (36.4)	Codominant	0.49 (0.28–0.86)	0.010
	Intron	T/A	78 (47.6)	19 (57.6)	Dominant	0.58 (0.26–1.31)	0.200
		T/T	47 (28.7)	2 (6.1)	Recessive	0.14 (0.03–0.63)	0.001
					Overdominant	1.70 (0.78–3.68)	0.180
NTRK3	rs1128994	C/C	105 (64.4)	19 (59.4)	Codominant	1.26 (0.65–2.43)	0.500
	Synonymous	T/C	53 (32.5)	11 (34.4)	Dominant	1.23 (0.56–2.70)	0.610
		T/T	5 (3.1)	2 (6.2)	Recessive	1.90 (0.34–10.62)	0.480
					Overdominant	1.10 (0.48–2.48)	0.830
NGFR	rs11466155	C/C	149 (90.8)	29 (87.9)	Codominant	1.46 (0.46–4.60)	0.530
	Synonymous	T/C	14 (8.5)	4 (12.1)	Dominant	1.56 (0.47–5.20)	0.480
		T/T	1 (0.6)	0 (0)	Recessive	0.00 (0.00-NA)	0.690
					Overdominant	1.62 (0.48–5.43)	0.450

*Total numbers of SNP differ because the genotypes of some SNPs were not determined.

†As the allele frequencies of *NTRK1* rs2150906 that did not have all three genotypes present in the study population, only allele frequencies were compared by the Chi-square test. And they were taken into account of Bonferroni's correction.

velopment in the postinductive stage, and inhibition of NT expression inhibits kidney morphogenesis (29,30). Some recent studies have shown strong expressions of NTs in diseased compared with normal human kidneys, especially for patients with GN and IgAN showed strong expressions of NTRs in the mesangial proliferation areas (24,31,32).

In terms of the renal disease progression, marked expressions of NGF and NGFR are reported in human proteinuric renal diseases (24), and the high levels of NGF expression in monocytes are associated with the presence of proteinuria in patients with GN. Furthermore, limited evidence exists for the involvement of NTs in the fibrotic processes as suggested by our data. NTs are expressed in renal interstitial fibroblasts (2,32) and they are found in the interstitial area near peritubular spaces and in the area of interstitial fibrosis, specifically in patients with IgAN, suggesting that NT systems could be associated with the pathogenesis of IgAN progression (24,31).

Although our data implicate SNPs of NTs and their receptors in the pathophysiology of IgAN, defining the exact function of these SNPs is difficult. In the analysis of our case-control study, *NGF* rs11102930 (promoter) was found to be associated with the susceptibility to childhood IgAN with a sufficient sample power. Moreover, associations between pathogenesis of IgAN and some candidate SNPs were found in the patient subgroup analysis: *NGF* rs6330 (missenese), *NTRK1* rs6334 (synonymous), *BDNF* rs11030104 (intron), *BDNF* rs7103411 (intron), *BDNF* rs7103873 (intron), and *BDNF* rs6484320 (intron).

As the missense SNPs are defined to make amino acid sequence changes (Ala to Val in *NGF* rs6330), they have enough possibilities to affect protein expression and its function. However, it is very difficult to explain the expected effects in the pathogenesis of intron and synonymous SNPs, which do not affect the amino acid sequences. Recently, it has been issued that synonymous SNPs might play an important role in the protein activities and specificities without influencing amino acid sequences (33,34). In addition, some introns have been found to affect various degrees of the efficiency of normal splicing and to structurally stabilize pre-mRNA to protect it against degradation, control the expression of exons (miRNA) to protect from degradation, and enhance protein production (35,36). However, it would be a more reasonable explanation that these SNPs might be linked with other coding SNPs as a haplotype to affect the phenotype in this case.

In terms of promoter SNPs, we investigated whether these genetic variants influence transcription factor binding sites to examine the transcription binding activity of three promoter SNPs (*NGF* rs11102930, *NTRK2* rs1187321, and *NTRK2* rs1187323) that showed significant association with the pathogenesis of IgAN. The transcription factor binding sites were compared using the online program "AliBaba 2.1" (http://www.gene-regulation.com/pub/programs/alibaba2). In the *NTRK2* rs1187321 site, two transcription factors (HNF-1, NF-ATc3, C/EBPa1p, Id3, and AFP1) can bind to the A-containing sequence, and only two (Oct-2.1 and HNF-1) to the T-containing sequence. In the *NGF* rs11102930 site, Sp1 can bind to the C, and no transcription factor to the T. However, sequence change in *NTRK2* rs1187323 (A to C) did not affect binding of transcription factors (Sp1). This result indicates that *NTRK2* rs1187321 and *NGF* rs11102930 have possibilities to influence protein expressions and/or their functions.

To our knowledge, there has been no report of an association between NT- and NTR-related polymorphisms and patients with GN. However, it is evident that NTs and NTRs could act as an important pathway during the development and progression of IgAN. Thus, we suggest taking a new insight into the neurotrophin pathway considering the pathogenesis of IgAN and it is necessary to investigate more SNPs of NT- and NTR-encoding genes.

In summary, we found that some SNPs of NT- and NTR-encoding genes were associated with susceptibility to IgAN and with the pathologic progression of IgAN, namely, the presence of proteinuria, renal disease progression markers, and podocyte foot process effacement.

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