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CLINICAL STUDY

MCP1 2518 A/G polymorphism affects progression of childhood focal segmental glomerulosclerosis

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Abstract

Monocyte chemoattractant protein-1 (MCP-1) is a highly specific chemokine for monocytes and plays roles in pathogenesis of various renal diseases. The aim of this study is to investigate the effect of *MCP1* 2518 A/G polymorphism on the incidence and clinical course of focal segmental glomerulosclerosis (FSGS) in children. *MCP1* 2518 A/G genotype was identified by PCR-RFLP in 60 biopsy-proven FSGS patients, 76 steroid sensitive nephrotic syndrome (SSNS) patients, and 96 healthy children. MCP-1 levels in urine and serum were measured by ELISA in all patients and the correlations of genotype with MCP-1 levels and clinical outcome were evaluated. The genotype frequencies for *MCP1* were similar in all groups. The percentage of patients who develop chronic renal failure was higher in patients with AA allele compared to GA or GG alleles (46% vs. 35% respectively, $p < 0.01$, Odds ratio: 1.59). Serum MCP-1 levels were similar in all groups, whereas urinary MCP-1 levels of the patients with FSGS (1680 pg/mg creatinine) were significantly higher than that of patients with SSNS (365 pg/mg creatinine, $p < 0.05$) and healthy controls (348 pg/mg creatinine; $p < 0.05$). Urinary MCP-1 levels were correlated with the degree of proteinuria in FSGS group ($r = 0.529$, $p = 0.016$). Our results suggest that the AA genotype might be a risk factor for the progression of renal disease in FSGS and *MCP1* genotyping may help the physicians to predict prognosis in these patients.

Keywords

Children, FSGS, MCP-1, nephrotic syndrome, polymorphism

History

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Introduction

Focal segmental glomerulosclerosis (FSGS) is an important cause of end-stage renal disease (ESRD) in children and characterized with poor response to corticosteroids.[1] Over the past several years, the incidence of FSGS in adults and children appears to be increasing.[2] There is increasing evidence that the genetic background of the adults and children with FSGS affects the progression of the disease and the response to therapy.[2,3]

Although patients with FSGS may present with any degree of proteinuria, clinical concern is greatest for those who have nephrotic-range proteinuria because without treatment, they have an extremely poor prognosis and mostly progress to ESRD in 3–6 years.[4] It is widely recognized that the prognosis in nephrotic patients with primary FSGS is significantly improved if partial or complete remission of proteinuria is achieved.[5,6]

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Monocyte chemoattractant protein-1 (CCL2/MCP-1) is a chemokine that mediates renal interstitial inflammation, tubular atrophy, and interstitial fibrosis by recruiting monocytes–macrophages into renal interstitium.[7] In a previous study, it has been shown that the degree of urinary albumin/creatinine ratio was correlated with urine MCP-1 levels and interstitial macrophage infiltration in patients with chronic kidney disease.[8] *In vitro* experiments have also demonstrated that tubular epithelial cells release MCP-1 when exposed to serum proteins in the apical side.[9] Urinary MCP-1 levels have been shown to be positively correlated with the degree of proteinuria in children with FSGS and IgA nephropathy.[10] There are several studies reporting the relationship between *MCP1* 2518 A/G polymorphism and prognosis of various renal diseases such as IgA nephropathy,[11,12] diabetic nephropathy[13] and lupus nephritis,[14] however the association of this polymorphism with the clinical outcome in primary FSGS has not been determined yet. The aim of this study was to investigate the relationship between *MCP1* 2518 A/G polymorphism and urinary-serum MCP-1 levels with the incidence and clinical course of children with primary FSGS.

Methods

Patients

Sixty patients with biopsy-proven FSGS, 76 patients with steroid-sensitive nephrotic syndrome (SSNS), and 96 healthy children were included in the study. The study protocol was approved by the institutional ethical committee. Written informed consent for the genetic analysis was obtained from each family.

Clinical characteristics and laboratory findings including hematuria, hypertension, urinary protein/creatinine ratio, serum creatinine, albumin levels, and steroid sensitivity of the patients at the beginning and follow-up period were collected by standard clinical questionnaire forms. Serum and urine MCP-1 levels of the patients were measured during the active state of the disease (i.e. during nephrotic range proteinuria).

Inclusion criteria for FSGS group were to have biopsy-proven disease with age at disease onset between 1 and 16 years and the absence of any known cause for secondary FSGS. The inclusion criteria for SSNS group were to be responsive to initial standard 4 weeks prednisolone therapy with age at disease onset between 1 and 8 years and to have histopathologically minimal change disease for those patients who underwent a renal biopsy for any reason. Healthy controls had no personal or family history of renal disease and hypertension.

MCP1 Genotyping

Genomic DNA was extracted from peripheral leukocytes by using standard procedures. The *MCP1* genotypes were identified by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) assay using *PvuII* restriction enzyme (Life Technologies, Carlsbad, CA). A 930-bp fragment including the distal regulatory segment of the *MCP1* 5′-flanking region (nucleotides −2746 to −1817) was analyzed. Primers used were 5′-CCG AGA TGT TCC CAG CAC AG-3′ (forward) and 5′-CTG CTT TGC TTG TGC CTC TT-3′ (reverse). PCR products were digested by *PvuII* and the products were separated by gel-electrophoresis. Samples with a single 930-bp band were identified as AA, samples with three bands of 930, 708, and 222 bp were typed as AG, and those with two bands of 708 and 222 bp were typed as GG.

MCP-1 Measurements

Serum and urinary MCP-1 levels were measured by solid-phase enzyme linked immunosorbent assay (ELISA) in all patients (Quantikine MCP-1 ELISA; R&D Systems, Minneapolis, MN). All assays were done in duplicate. The mean variation in each sample was <5%. The minimum detectable MCP-1 level with this kit is 5 pg/mL. There is no cross-reactivity with the closely related chemokines MIP-1α, MIP-1β, and MCP-2,-3, and -4. Urine MCP-1 levels were normalized according to urine creatinine levels.

Statistical analysis

The results are presented as mean ± standard deviation (SD) unless otherwise specified. Mann–Whitney *U* test for paired samples was used for comparison between groups. Correlations were calculated with Pearson product moment correlation coefficient. $p < 0.05$ was considered statistically significant.

Results

The characteristics of children with FSGS and SSNS are demonstrated in Table 1. Control group was age (72.3 ± 39.9 months) and sex (40 males, 36 females) matched with the FSGS group. Mean age at the diagnosis was higher in FSGS group than that in SSNS group ($p < 0.01$, Table 1). Five patients in FSGS group had hypertension at initial presentation whereas none in SSNS group. Serum albumin and creatinine levels were similar in both groups. Microscopic hematuria was a more common finding in the FSGS group ($p < 0.01$, Table 1). The urinary protein/creatinine ratio in SSNS group was higher than that in FSGS group ($p < 0.01$, Table 1). In both groups, mean follow-up duration was about 5.5 years. During the follow-up period, renal functions of all patients with SSNS remained stable whereas 25 patients (41.6%) in FSGS group developed chronic renal failure (Table 1).

The *MCP1* genotypes of all groups are presented in Table 2. Genotype and allel frequencies were comparable in all groups ($p > 0.05$). The *MCP1* genotype had no effect on age at diagnosis, hypertension, degree of proteinuria, and steroid sensitivity (data not shown). The FSGS patients who progressed to chronic renal failure or who had stable renal functions during the follow-up were comparable in terms of

Table 1. Clinical and laboratory features of the patients with FSGS and SSNS at initial presentation and the disease course.

Clinical and laboratory features	FSGS group ($n = 60$)	SSNS group ($n = 76$)
Age at the diagnosis (months)	75.6 ± 47.7 (10–201)**	39.9 ± 31.1 (10–146)
Sex (Male/Female)	35/25	42/34
Hypertension	5 (8.3%)	–
Steroid sensitivity	4 (6.7%)**	76 (100%)
Serum albumin (g/dl)	2.1 ± 0.8 (0.9–4.0)	1.8 ± 0.4 (1.1–2.9)
Serum creatinine (mg/dl)	0.3 ± 0.2 (0.1–0.5)	0.5 ± 0.3 (0.2–1.2)
Microscopic hematuria	18 (30.0%)**	1 (1.3%)
Urinary protein/creatinine (mg/mg)	3.5 ± 3.1 (1.2–17.0)**	10.3 ± 4.6 (5.9–16.0)
Follow-up duration (month)	66.4 ± 49.6 (6–219)	67.1 ± 59.2 (6–226)
Chronic renal failure	25 (41.6%)	–

Notes: Data are presented as mean ± SD with range or percentage in parenthesis where appropriate.
**$p < 0.01$ compared to SSNS group.

age at diagnosis, sex, prevalence of hypertension, and serum creatinine level at presentation (data not shown). The percentage of patients who develop chronic renal failure was significantly higher in AA genotype compared to GA and GG genotype (46% vs. 35% respectively, $p<0.01$, Odds ratio: 1.59, Table 3).

There was no correlation between urinary and serum MCP-1 level in all groups, but a positive correlation was found between urinary MCP-1 level and urinary protein/creatinine ratio in FSGS group ($r=0.529$, $p=0.016$). There was no association between serum MCP-1 level and degree of proteinuria. Serum MCP-1 levels were similar in all groups, however urinary MCP-1 levels of FSGS group were higher than SSNS group ($p<0.05$, Table 4) and healthy controls ($p<0.05$, Table 4). There was no correlation between *MCP1* genotype and urine or serum MCP-1 level.

Discussion

Clinical course of FSGS is highly variable and correlated with severity of interstitial inflammation, which plays an important role in progression of the disease. Recruitment of monocytes/macrophages by MCP-1 and other chemokines to interstitium is a key process, which leads to chronic inflammation and eventually glomerular sclerosis.[15,16] Previous studies showed the relationship between *MCP1* 2518A/G polymorphism and severity of various renal diseases.[12–14] In this study, we investigated for the first time the relationship between *MCP1* 2518A/G polymorphism and disease course in a group of FSGS patients.

MCP1 2518 A/G polymorphism affects a region that regulates the transcription of this gene.[17] This polymorphism was originally described to affect MCP-1 production by peripheral mononuclear cells in response to an inflammatory stimulus.[18] The effect of the presence of A or G alleles at this position on MCP-1 production is controversial. Rovin et al. reported that G at 2518 position causes increased in vitro MCP-1 production by mononuclear cells in response to IL-1β.[18] However, Kim et al. reported that A at 2518 position was associated upregulation of *MCP1* and results in severe proteinuria in patients with lupus nephritis.[19] We demonstrated that the *MCP1* genotype frequencies for AA, GA and GG were similar in FSGS, SSNS, and control groups, which suggest that *MCP1* 2518 genotype is not a risk factor for the development of FSGS. However, the percentage of patients who progressed to chronic renal failure was significantly higher in those carrying AA genotype when compared to those who had GA or GG genotypes. This observation may suggest that *MCP1* 2518 A/G polymorphism might decrease the risk of progression to chronic renal failure in FSGS patients. However, this polymorphism seems to have no effect on the clinical parameters at the presentation and severity of proteinuria. Similarly, Steinmetz et al.[11] reported that 2518 A/G polymorphism had no effect on the incidence and progression of IgA nephropathy in a group of Caucasian patients with a mean follow-up period of 45.5 months. However, although not statistically significant, they also noted that the incidence of end-stage renal disease in patients with the GG genotype was lower than that in patients with the AA or AG genotype. Mori et al.[12] performed a similar study in Japanese patients with IgA nephropathy. They found that the AA genotype was an independent risk factor for the progression of the IgA nephropathy, and was closely associated with renal survival. This discrepancy may be explained by ethnicity and also the duration of follow-up, which was longer (average 135.9 months) in the latter study.

Table 4. Urinary CCL2/MCP-1 levels in patients with focal segmental glomerulosclerosis and SSNS, and control group.

		Serum MCP-1 (pg/mL)		Urinary MCP-1 (pg/mg creatinine)	
Groups	*n*	Median	IQR	Median	IQR
FSGS	60	359	493	1680*#	2282
SSNS	76	414	896	365	408
Control	96	590	492	348	412

Notes: *$p<0.05$ compared to SSNS group, #$p<0.05$ compared to Control group. FSGS: Focal segmental glomerulosclerosis; SSNS: Steroid sensitive nephrotic syndrome; CCL2/MCP-1: Monocyte chemoattractant protein-1.

Table 2. *MCP1* 2518 genotypic and allele frequencies of patients with FSGS, SSNS and healthy controls.

	MCP1 2518 Genotype			Alleles	
	AA n (%)	GA n (%)	GG n (%)	A n (%)	G n (%)
FSGS group ($n=60$)	37 (61)	19 (32)	4 (7)	93 (78)	27 (22)
SSNS group ($n=76$)	40 (53)	31(41)	5 (6)	111 (73)	41 (27)
Healthy controls ($n=96$)	55 (57)	38 (40)	3 (3)	148 (77)	44 (23)

Note: Data are presented as patient numbers with percentage in parenthesis.

Table 3. The genotypic frequencies in patients with and without chronic renal failure in FSGS group.

	MCP1 2518 Genotype			Alleles	
Patients	AA *n* (%)	GA *n* (%)	GG *n* (%)	A *n* (%)	G *n* (%)
Patients with CRF ($n=25$)	17 (68)	5 (20)	3 (12)	39 (78)	11 (22)
Patients without CRF ($n=35$)	20 (57)	14 (40)	1 (3)	54 (77)	16 (23)

Note: Data are presented as patient numbers with percentage in parenthesis. CRF: Chronic renal failure.

MCP1 2518 A/G polymorphism affects the promoter region of the gene and the effect of this polymorphism on transcription in FSGS patients is yet to be determined.

Increased urinary MCP-1 levels have been associated with disease activity and poor prognosis in various renal diseases.[8] In our study, serum MCP-1 levels of FSGS group were comparable with the healthy controls and there was no correlation between urinary and serum MCP-1 levels in all study groups. Our findings suggest that increased urinary excretion of MCP-1 in the patients with FSGS is most likely due to enhanced production of MCP-1 in kidney, presumably induced by excessive exposure to plasma proteins filtered from the damaged glomeruli. We also showed that urinary MCP-1 levels correlated with degree of proteinuria in FSGS patients. Similarly, previous studies reported the increased urinary MCP-1 levels and the correlation between degree of proteinuria and urinary MCP-1 levels in patients with FSGS and IgA nephropathy.[10] A number of studies have demonstrated a correlation between degree of proteinuria and progression rate of chronic kidney disease, which have led to the hypothesis that proteinuria might be an independent player for disease progression rather than simply being a marker of glomerular dysfunction.[20–22] It has also been demonstrated that high protein load in renal tubular cells up-regulates MCP-1 expression and thereby MCP-1 production.[9,23] Interestingly, despite more severe proteinuria in SSNS group, the patients with FSGS had higher urinary MCP-1 levels. This finding suggests that the role of MCP-1 in pathogenesis of FSGS is not a direct result of proteinuria. Proteinuria itself may be a result of immunological insult to glomeruli, in which MCP-1 plays a critical role in this scenario. It has been shown that podocytes can be detected in urine specimens of FSGS patients but not in healthy controls or minimal-change nephrotic syndrome patients.[24] Inflammatory cytokines such as TGF-β is overexpressed in kidney biopsy specimens of FSGS patients[25] and cultured podocytes express MCP-1 in presence of TGF-β.[26,27] Thus, another explanation for the increased urinary MCP-1 levels in FSGS patients may be the presence of MCP-1 expressing podocytes in urine of these patients but not in SSNS or control groups. The major limitation of the current study is limited patient number. Further studies investigating the role of *MCP-1* genotype in a larger group of FSGS patients and functional studies investigating the effect of *MCP-1* 2518 A/G polymorphism on proteinuria and MCP-1 production by podocytes and macrophages may help to clarify the role of MCP-1 in this disease.

In conclusion, AA allele might be a risk factor for disease progression in FSGS and *MCP-1* genotyping may be useful in these patients to predict the prognosis. This approach might also guide the physicians to determine the patients who need more aggressive treatment.

Declaration on interest

The authors report no conflict of interest. This study was supported by The Scientific and Technological Research Council of Turkey [Grant number: SBAG2685(103S024)].

References

1. Gbadegesin R, Lavin P, Foreman J, Winn M. Pathogenesis and therapy of focal segmental glomerulosclerosis: an update. *Pediatr Nephrol.* 2011;26(7):1001–1015.
2. Eddy AA, Symons JM. Nephrotic syndrome in childhood. *Lancet.* 2003;362(9384):629–639.
3. Machuca E, Benoit G, Antignac C. Genetics of nephrotic syndrome: Connecting molecular genetics to podocyte physiology. *Hum Mol Genet.* 2009;18(R2):R185–R194.
4. Cattran DC, Rao P. Long-term outcome in children and adults with classic focal segmental glomerulosclerosis. *Am J Kidney Dis.* 1998; 32(1):72–79.
5. Gipson D, Chin H, Presler T, et al. Differential risk of remission and ESRD in childhood FSGS. *Pediatr Nephrol.* 2006; 21(3):344–349.
6. Troyanov S, Wall CA, Miller JA, Scholey JW, Cattran DC, Toronto Glomerulonephritis Registry Group. Focal and segmental glomerulosclerosis: definition and relevance of a partial remission. *J Am Soc Nephrol.* 2005;16(4):1061–1068.
7. Yadav A, Saini V, Arora S. MCP-1: Chemoattractant with a role beyond immunity: A review. *Clin Chim Acta.* 2010;411(21–22):1570–1579.
8. Eardley KS, Zehnder D, Quinkler M, et al. The relationship between albuminuria, MCP-1//CCL2, and interstitial macrophages in chronic kidney disease. *Kidney Int.* 2006; 69(7):1189–1197.
9. Burton CJ, Combe C, Walls J, Harris KPG. Secretion of chemokines and cytokines by human tubular epithelial cells in response to proteins. *Nephrol Dial Transpl.* 1999; 14(11):2628–2633.
10. Wasilewska A, Zoch-Zwierz W, Taranta-Janusz K, Kołodziejczyk Z. Urinary monocyte chemoattractant protein-1 excretion in children with glomerular proteinuria. *Scand J Urol Nephrol.* 2011;45(1):52–59.
11. Steinmetz OM, Panzer U, Harendza S, et al. No association of the–2518 MCP-1 A/G promoter polymorphism with incidence and clinical course of IgA nephropathy. *Nephrol Dial Transpl.* 2004; 19(3):596–601.
12. Mori H, Kaneko Y, Narita I, et al. Monocyte chemoattractant protein-1 A-2518G gene polymorphism and renal survival of Japanese patients with immunoglobulin A nephropathy. *Clin Exp Nephrol.* 2005;9(4):297–303.
13. Su N, Li HY, Huang MF, Jiang ZP, Zhou TB. Association of monocyte chemoattractant protein-1 2518G/A gene polymorphism with diabetic nephropathy risk. *J Recept Signal Transduct Res.* 2014;35(1):94–97.
14. Malafronte P, Vieira JM, Pereira AC, Krieger JE, Barros RT, Woronik V. Association of the MCP-1−2518 A/G polymorphism and no association of its receptor CCR2−64 V/I polymorphism with lupus nephritis. *J Rheumatol.* 2010; 37(4):776–782.
15. Vielhauer V, Berning E, Eis V, et al. CCR1 blockade reduces interstitial inflammation and fibrosis in mice with glomerulosclerosis and nephrotic syndrome. *Kidney Int.* 2004; 66(6):2264–2278.
16. Kim MJ, Tam FWK. Urinary monocyte chemoattractant protein-1 in renal disease. *Clin Chim Acta.* 2011;412(23–24):2022–2030.
17. Ueda A, Okuda K, Ohno S, et al. NF-kappa B and Sp1 regulate transcription of the human monocyte chemoattractant protein-1 gene. *J Immunol.* 1994;153(5):2052–2063.
18. Rovin BH, Lu L, Saxena R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. *Biochem Biophys Res Commun.* 1999;259(2):344–348.
19. Kim HL, Lee DS, Yang SH, et al. The polymorphism of monocyte chemoattractant protein-1 is associated with the renal disease of SLE. *Am J Kidney Dis.* 2002;40(6):1146–1152.
20. Schaefer B, Wühl E. Educational paper: Progression in chronic kidney disease and prevention strategies. *Eur J Pediatr.* 2012; 171(11):1579–1588.
21. Ishikura K, Uemura O, Hamasaki Y, et al. Progression to end-stage kidney disease in Japanese children with chronic kidney disease: results of a nationwide prospective cohort study. *Nephrol Dial Transpl.* 2014;29(4):878–884.

22. Fathallah-Shaykh SA, Flynn JT, Pierce CB, et al. Progression of pediatric CKD of nonglomerular origin in the CKiD cohort. *Clin J Am Soc Nephrol.* 2015;10(4):571–577.
23. Lai KN, Leung JCK, Chan LYY, Guo H, Tang SCW. Interaction between proximal tubular epithelial cells and infiltrating monocytes/T cells in the proteinuric state. *Kidney Int*. 2007; 71(6):526–538.
24. Nakamura T, Ushiyama C, Suzuki S, et al. The urinary podocyte as a marker for the differential diagnosis of idiopathic focal glomerulosclerosis and minimal-change nephrotic syndrome. *Am J Nephrol.* 2000;20:175–179.
25. Kim JH, Kim BK, Moon KC, Hong HK, Lee HS. Activation of the TGF-β/Smad signaling pathway in focal segmental glomerulosclerosis. *Kidney Int.* 2003; 64(5):1715–1721.
26. Lee EY, Chung CH, Khoury CC, et al. The monocyte chemoattractant protein-1/CCR2 loop, inducible by TGF-β, increases podocyte motility and albumin permeability. *Am J Physiol Renal Physiol.* 2009;297:F85–F94.
27. Lee HS. Mechanisms and consequences of TGF-β overexpression by podocytes in progressive podocyte disease. *Cell Tissue Res.* 2012;347(1):129–140.