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Research article

CCR2 V64I Genotyping: Impact on end stage renal disease development, progression and renal transplantation outcome

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Key words: CCR2 V64I genotypes, ESRD, Renal graft rejection, Hemodialysis, RFLP, Gene polymorphism, Children, Renal Transplantation.

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Abstract

Chemokine receptor 2 (CCR2) may have an impact on end stage renal disease (ESRD) development in children as well as renal allograft survival. **Objective:** Detection of the relevance of the *CCR2 V64I* gene polymorphism to the development and progression of ESRD and its impact on graft rejection in transplanted children. **Methods:** Genotyping for *CCR2 V64I* was done for seventy five children with end-stage renal disease (ESRD) [50 treated with renal transplantation and 25 with hemodialysis] and seventy five healthy children by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) analysis. **Results:** The *CCR2 V64I* displayed significantly higher frequencies among transplantation, hemodialysis, ESRD-patients as well as those with acute rejection when compared with the control subjects (P value <0.001for all).The mutant A allele displayed statistically significant frequencies in all groups when compared with the control group (P value < 0.001). Moreover, carriers of mutant A allele had increased risk of developing both ESRD and acute rejection after transplantation [32.4 times more risk to develop ESRD (OR 32.4; 95% CI 14.1-74.1, P value <0.001) and 5.1 times more risk to suffer acute graft rejection (OR 5.1; 95% CI 1.6-16.1, P value 0.03)]. **Conclusion:** The frequency of the A allele of the *CCR2 V64I* genotypes was significantly higher among children with ESRD & those with acute graft rejection and this allele might be considered a risk marker for pediatric ESRD development as well as a predictor of graft rejection.

Introduction

End-stage renal disease (ESRD) is considered a major pediatric health obstacle affecting about 5 to 10 per million children every year [1]. For these children, successful kidney transplantation provides better quality of life and longer survival than dialysis [2].

In spite of advances in immunosuppressive modalities as well as the overall medical care of renal transplant recipients with consequent improvement in allograft survival, yet chronic renal allograft rejection remains a critical impediment to successful organ transplantation with acute rejection representing the most important risk factor for chronic renal allograft rejection [3].

Patient's immunologic responses play pivotal roles in the original kidney disease pathogenesis, progression, influencing the modality and success of its therapy as well as the recurrence of underlying kidney disease [4].

Immunological responses mediated by chemokine/ chemokine receptor have been embroiled in the pathogenesis of renal disease as well as the survival of renal graft after transplantation. The interactions of chemokines and their corresponding receptors initiate signaling pathways through which tissue maintenance, wound healing and infection could occur. Chemokine ligand2 (CCL2)/monocyte chemo-attractant protein-1 (MCP-1) is produced by macrophages and endothelial cells and upon its engagement with the corresponding receptor, chemokine receptor 2 (CCR2), they stimulate chemotaxis of monocyte/macrophages as well as other inflammatory cells [5].

Further recruitment of these cells will be implicated in their adherence to the endothelial cells and over expression of pro-inflammatory mediators e.g. lysosomal enzymes, reactive oxygen species and nitric oxide as well as transforming growth factor-beta and vascular endothelial growth factor [6]. These mediators could play fundamental roles in the development and progression of native as well as graft kidney damage i.e. CCL2/CCR2 leukocyte recruitment at site of inflammation has been also implicated in transplant rejection [7].

With choice of transplantation as a modality for renal replacement therapy in these children, the differences in the outcome might be attributed to genetic polymorphisms of genes other than those at the HLA locus, and these polymorphisms could be considered as

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excellent parameters that might explain such heterogeneity. Numerous genetic variations have been specified in a number of gene encoding molecules participating in the recipient's immune response to the renal allograft [4].

CCR2 gene is located on chromosome 3p21 within a cluster of chemokine receptor genes. Several polymorphisms could be detected in *CCR2* gene, among them is a single nucleotide polymorphism (SNP) of guanine to adenine at position 190 of *CCR2* gene changing the amino acid valine to isoleucine at position 64, within the first transmembrane domain of this protein [8].

This study intended to detect the impact of *CCR2 V64I* gene polymorphism on the development of ESRD and wither this mutation influence the success and survival of renal graft after transplantation or not?

Subjects and method

The study included 150 Egyptian subjects classified as:

Fifty children who had received a renal allograft at the Center of Pediatric Nephrology and Transplantation (CPNT), Cairo University Children's Hospital,, Egypt. All of them had received their first graft. Age, gender, duration of dialysis, as well as the donor data were recorded. Patients were reviewed during their routine follow up at the Pediatric Nephrology Clinic.

Twenty-five pediatric patients with advanced chronic kidney disease (CKD) [stage 5] based on estimated glomerular filtration rate (eGFR) according to the National Kidney Foundation classification [9] were also included in the study, selected from the Hemodialysis Unit at CPNT**.** The inclusion criteria for hemodialyzed patients included: onset of hemodialysis below 18 years with at least 6 months duration. Patients received HD for 4 hours three times weekly with a polysulfone membrane using bicarbonate-buffered dialysate. Children who received hemodialysis for less than 6 months were excluded.

Seventy-five healthy children attended the pediatric clinic of The Medical Research Center of Excellence (MRCE) of the National Research Centre (NRC), with no clinical signs of renal disease and no family history of renal disease served as controls.

The study was done from March 2014 to December 2017. An informed consent for genetic studies was obtained from parents of all participants. The protocol of the study was read and approved by the Ethics Committee of NRC in Egypt.

Diagnostic criteria for acute organ rejection were: sudden decrease in urine output, fever, and abdominal tenderness accompanied by increased serum creatinine and urea nitrogen, decreased or unchanged urine specific gravity, hematuria and proteinuria. In addition, ultrasound examination showing increased kidney volume (with or without decreased blood flow), and an increased blood flow index. Acute rejection which is cellular rejection due to T cell activation encountered in the first week after post-transplant was defined and graded according to the Banff Criteria [10]. It was defined as either borderline/suspicious or acute rejection in patients with stable serum creatinine values at the time of biopsy [grades 3 and 4] [11]. No protocol biopsies were performed, particularly as the patients were pediatric patients where invasive biopsy accrues more cost and risk than in the adult population. Renal biopsies were only performed if there were clinical indications with suspicion for allograft dysfunction.

Organ recipients showing signs of chronic calcineurin inhibitor (CNI) nephrotoxicity, acute tubular necrosis, ureteral obstruction and/or renal artery stenosis of the graft, arterial and venous thrombosis, and infection-induced fever were excluded from the study. Fourteen patients were diagnosed with organ rejection and the diagnosis confirmed by renal graft biopsies. Timing of acute rejection in our patients ranged from one day to 16 months post transplantation, with 52% of rejections occurring in the first 6 months post-transplantation.

Initial FK506 dose was 0.16 mg/kg per day by oral route (1.5-6 mg/day), and target trough levels were 3-14 ng/mL in the first 3 months and 4.5 ng/mL in the FK506/ everolimus group. Initial dose of mycophenolate mofetil (MMF) was 360-1440mg/day, and dose was modified based on adverse effects such as diarrhea or leucopenia. IL-2 receptor blocking antibody (anti-IL-2R Ab, Basiliximab) (Simulect, Novartis Pharmaceuticals, Basel, Switzerland), was given to 10 patients (BSX group) (CsA or FK506 based immunosuppression) 4 hrs before and 3 days after renal transplantation (two 10 mg doses for patients weighing less than 35 kg, and two 20-mg doses for patients weighing more than 35 kg). Anti-thymocyte globulin (ATG) (Thymoglobulin, Genzyme Transplant, Cambridge, MA) was given to 29 patients (THYMO group) as a single dose of 5-8 mg/kg on transplantation day (Day 0). Everolimus was administered 2 mg per day and Sirolimus was loaded 6 mg per day and then adjusted dose of 2 mg/day was maintained with target trough level of 5-15 ng/mL.

A peripheral blood sample was obtained from all subjects. An immediate centrifugation was done for 10 min at 5000 rpm at 4°C. All samples were stored at−20 °C until assay. One ml of venous blood sample was collected on EDTA vials, for the extraction of genomic DNA.

The following parameters were measured: urea, calcium, phosphorus and albumin by routine methods using (Olympas AU 400 : Olympus diagnostic, Japan).

CCR2 V64I genotyping

Genomic DNA was extracted from EDTA–anticoagulated whole blood samples using the QIAamp DNA Mini isolation kit (QIAGEN, #51304, Germany) following

manufacturer's instructions and was stored at−20 °C until the analysis.

Genotype was determined by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) analysis.

The PCR mixture in a 25-μL final volume consisted of 12.5 μL PCR master mix (Fermentas, St. Leon-Rot, Germany), 9.5 μL ddH2O, 1 μL of each primer, and 1 μL DNA. The G to A mutation at position 190 of *CCR2* gene was determined by PCR-RFLP. The following primers were used for amplification:

forward 5′-CAT TGC AAT CCCAAA GAC CCA CTC-3′ and

reverse 5′-TTG GTT TTG TGG GCA ACA TGA TGG-3′.

Initial denaturation at 94 °C for 5 minutes was followed by 33 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds and extension at 72 °C for 30 seconds. Final extension step was at 72 °C for 5 minutes. The PCR product (5 μl) was digested for 2 hours at 65 °C with 2.5 U of BsaBI restriction endonuclease (Fermentas). Digestion products were analyzed by electrophoresis on 3% agarose gel in TBE buffer and visualized using ethidium bromide staining. Samples with a single 173bp band were identified as having GG genotype, samples with two bands, 149 bp and 24 bp as AA genotype and those with three bands, 173 bp, 149 bp and 24 bp as GA heterozygotes [12].

Statistical analysis

Data were analyzed using SPSS© Statistics version 17 (SPSS Inc., Chicago, IL). Parametric numerical variables were presented as mean ± SD and intergroup differences were compared using *one-way* analysis of variance (*ANOVA*). Non-parametric numerical variables were presented as median and interquartile range/min and max. Categorical variables were presented as number and percentage and intergroup differences were compared using the chi squared test and odds ratio (OR) with a 95% confidence interval (95% CI) for trend. P-value <0.05 was considered statistically significant.

Results

As mentioned before this study included 75 ESRD patients, 50 of them [35 (70%) males & 15 (30%) females] were treated by renal transplantation and aged 12.6±3.4 years. The remaining 25 [9 (36%) males & 16 (64%) females] were treated with hemodialysis and aged 8.1±3.6 years. These patients developed ESRD 2.5± 1.3 years after the onset of original kidney disease. The study included another 75 healthy children [42 (56 %) males & 33 (44%) females] as controls with age 9.7± 3.4 years. Also, 50 living healthy subjects volunteered to serve as kidney donors [22 males (44%) and 28 females (56%)] with an average age of 37±6.4 years). The clinical and biochemical characteristics of the studied groups are shown in table 1. There were statistically significant differences regarding predialysis systolic (SBP) & diastolic (DBP) blood pressure as well as predialysis blood urea (P value <0.001 for all)

Upon comparing the clinical characteristics of the whole 75 ESRD-children (44 males & 31 females with mean age 11.0 ± 4.0 years) with the control group, there were statistical significances regarding serum phosphorus (4.6 ±1.2 vs. 3.4 ±0.6, P value <0.001) and albumin levels (3.9 ±0.5 vs. 4.3 ±0.5, P value <0.001).

Table 1. Basal characteristics of the studied groups.

	Controls (n= 75)	Renal transplantation group (n= 50)	Hemodialysis group (n= 25)	P-value
Duration of dialysis	NA	18.0(1.0- 114.0)	48.0(1.0- 72.0)*	0.025
Predialysis – SBP (mmHg)	113 ± 4.7	109.4 ± 10.5	126.4 ± 18.5	< 0.001
Predialysis – DBP (mmHg)	73.5 ± 4.8	70.4 ± 8.9	78 ± 9.1	< 0.001
Predialysis-urea (mg/dl)	13.5±3.1	20.3±11.8	80.2±9.1	< 0.001
Calcium (mg/dl)	9.4 ± 0.5	9.5 ± 0.9	9.4 ± 1.1	0.5
Phosphorus (mg/dl)	3.4±0.6	4.5±0.7	4.7±1.8	0.4
Albumin (g/dl)	4.3 ±0.5	3.9 ± 0.5	3.9 ± 0.6	0.7

Data are presented as mean ±SD, * median (min- max).

The *CCR2 V64I* genotype and allele frequencies were compared among the three studied groups and presented in table 2. Statistically significant differences were demonstrated regarding the *CCR2 V64I* genotypes between the three groups, where the GA+AA genotypes frequencies were higher in the patients treated by transplantation and hemodialysis compared to the control subjects (90%, 88% & 7% respectively, P value <0.001). Meanwhile the frequency of GG genotype was higher in the control group than the other two groups (90.7%, 10%, 12 respectively, P value <0.001). Also, The frequencies of A & G alleles differed significantly between groups, where A allele presented a higher frequency among the transplantation and hemodialysis groups when compared with the control group while the frequency of G allele was higher among the control group when compared with the other two groups (60%, 64%, 4.7% for A allele &

40%, 36%, 95.3% for G allele respectively, P value < 0.001).

The *CCR2 V64I* genotype frequencies and allele frequencies as well as the risk association were compared between ESRD-patients and controls and presented in table 3. The *CCR2 V64I* genotypes frequencies differed significantly between the two groups, where the frequencies of GA+AA genotypes were higher in the ESRD-children when compared to the control subjects, while the frequency of GG genotype was higher in the control group in comparison with ESRD-children (89.3% vs. 9.3% and 90.7% vs. 10.7% respectively, P value <0.001). Likewise, the A and G alleles frequencies differed significantly, where the A allele showed higher frequency among the ESRD-children group & the G allele frequency was higher among the control subjects (61.3 vs. 4.7% & 95.3% vs. 38.7%, P value < 0.001). As regards the demonstration of the risk association of the different *CCR2 V64I* genotypes and alleles with ESRD among the studied groups, GA +AA genotypes carriers have 81.4 times more risk to develop ESRD than GG genotype carriers (OR 81.4; 95% CI 27.9-237, P value < 0.001). Furthermore, children carrying the mutant A allele have 32.4 times more risk to develop ESRD when compared with wild G allele carriers (OR 32.4; 95% CI 14.1-74.1, P value < 0.001).

Patients with ESRD who were treated with renal transplantation were further classified into 2 groups:

Fourteen patients with acute graft rejection [rejection (+)] (9 males & 5 females) with age 13± 2.6 years.

Thirty six not suffering acute rejection after renal transplantation [rejection (-)] (26 males & 10 females) with age 12.2± 3.5.

The basal characteristics of both groups are shown in table 4.

The *CCR2 V64I* genotype frequencies and allele frequencies as well as the risk association were compared between renal transplantation recipients with and without acute rejection and presented in table 5. Statistically significant differences were demonstrated regarding the *CCR2 V64I* genotypes between the two groups (71.4% vs. 13.9% for AA genotype, 28.6% vs. 72.2% for GA genotype & Zero % vs. 13.9% for GG genotype; P value < 0.001). Also, the A and G alleles frequencies differed significantly where higher frequency of A allele was displayed among the rejection (+) renal transplant recipients& the frequency of G allele was higher among the rejection (-) renal transplant recipients group (85.7 vs. 54.2% & 45.8% vs. 14.3%, P value 0.03). Demonstration of the risk association of the different *CCR2 V64I* alleles with acute graft rejection among renal transplant recipients showed that carriers of mutant A allele have 5.1 times more risk to suffer acute graft rejection when compared with the wild G allele carriers (OR 5.1; 95% CI 1.6-16.1, P value 0.03).

Table 2. The *CCR2 V64I* genotypes & alleles frequencies and risk association among the studied groups.

Gene	Transplantation (n=50)	Hemodialysis (n=25)	Control (n=75)	P-value
Genotypes				
AA	15 (30%)	10 (40%)	Zero (0%)	
GA	30 (60%)	12 (48%)	7 (9.3%)	<0.001
GG	5 (10%)	3 (12%)	68 (90.7%)	
GA+AA	45 (90%)	22 (88%)	7 (9.3%)	
GG	5 (10%)	3 (12%)	68 (90.7%)	<0.001
Alleles	**(n=100)**	**(n=50)**	**(n=150)**	
A	60 (60%)	32 (64%)	7 (4.7%)	<0.001
G	40 (40%)	18 (36%)	143 (95.3%)	

Data are presented as frequency (percentage). P<0.05 was statistically significant.

Table 3. The frequency distribution and risk association of CCR2 V64Igenotypes and alleles among the studied groups.

Gene	ESRD-patients (n=75)	Controls (n=75)	*OR (95% CI)	P-value
Genotypes				
AA	25 (33.3%)	Zero (0%)		
GA	42 (56%)	7 (9.3%)		<0.001
GG	8 (10.7%)	68 (90.7%)		
GA+AA	67 (89.3%)	7 (9.3%)		
GG	8 (10.7%)	68 (90.7%)	81.4 (27.9-237)	<0.001
Alleles	**(n=150)**	**(n=150)**		
A	92 (61.3%)	7 (4.7%)		
G	58 (38.7%)	143 (95.3%)	32.4 (14.1-74.1)	<0.001

Data are presented as frequency (percentage). P<0.05 was statistically significant.

*** Odd's ratio was used.**

Table 4. Comparison of clinical parameters in children with and without acute rejection.

	Renal transplant recipients Rejection (+) (n= 14)	Renal transplant recipients Rejection (-) (n= 36)	P-value
Duration of dialysis*	30 (9.8-51.0)	18 (8.3-36.0)	0.12
Modality of dialysis			
Hemodialysis:	11 (78.6%)	30 (83.3%)	
Pre-emptive:	2 (14.3%)	6 (16.7%)	0.3
Peritoneal:	1 (7.1%)	Zero %	
Donor source			
Related:	13 (92.9%)	25 (69.4%)	0.08
Non related:	1 (7.1%)	11 (30.6%)	
Donor sex			
(male/female)	7 (50%) / 7 (50%)	15 (41.7%) / 21 (58.2%)	0.6
Number of mismatches			
1	2 (14.3%)	3 (8.3%)	
2	5 (35.7%)	13 (36.1%)	0.9
3	6 (42.9%)	18 (50%)	
4	1 (7.1%)	2 (5.6%)	
Ischemia time	52.5±16.4	52.5±10.4	1.0
SBP (mmHg)	109.9 ± 6.5	109.2±11.7	0.9
DBP (mmHg)	72.1±7.0	69.7±9.5	0.4
Pre-dialysis urea (mg/dl)	18.1±7.8	21.2±12.9	0.4
Calcium (mg/dl)	9.4 ± 0.9	9.5 ± 0.9	0.8
Phosphorus (mg/dl)	4.5±0.7	4.5±0.7	0.8
Albumin (g/dl)	3.9±0.5	3.9±0.5	0.9
Chronic nephropathy			
Present	4 (28.6%)	3 (8.3%)	0.06
Absent	10 (71.4%)	33 (91.7%)	

Data are presented as percentage, mean ±SD .*values are presented as median (interquartile range).

Table 5. The frequency distribution and risk association of *CCR2 V64I* genotypes and alleles among renal transplant recipients with and without acute rejection.

Gene	Rejection (+) (n=14)	Rejection (-) (n=36)	*OR (95% CI)	P-value
Genotypes				
AA	10 (71.4%)	5 (13.9%)		
GA	4 (28.6%)	26 (72.2%)		<0.001
GG	Zero	5 (13.9%)		
Alleles	(n=28)	(n=72)		
A	24 (85.7%)	39 (54.2%)	5.1 (1.6-16.1)	0.03
G	4 (14.3%)	33 (45.8%)		

*** Odd's ratio was used. Data are presented as frequency (percentage).**

Discussion

CCR2 is claimed to be the main chemokine receptor promoting macrophage and monocyte recruitment to sites of inflammation and is also expressed on T cells [13]. It is mainly produced by memory T cells, B cells, eosinophils, monocytes and dendritic cells [14].

The majority of tissue macrophages are derived from monocytes, especially monocytes expressing the chemokine receptor CCR2 [15]. In the kidney these cells promote immunological responses and act as key players in renal inflammation, injury and fibrosis. The heterogeneity of macrophage infiltration is clearly evident as they modulate not only injury, necrosis and apoptosis but also tissue repair [16]. The C–C motif CCR2 expressed on a monocytes subset participate in the battle of infection defense as well as chronic inflammation shedding the light on the duel impact of CCR2 in the renal disease pathogenesis by both promoting & limiting kidney disease progression [17].

In addition to leukocyte migration to sites of tissue injury, CCL2/CCR2 have many other functions including hematopoiesis, angiogenesis, homeostatic functions in leukocyte development and cell trafficking during immune surveillance [18].

Although ERSD development is a consequence of many etiological factors, yet the inflammatory cytokines & immune dysregulation still have a fundamental role in its pathogenesis since these immune modulators both initiating the kidney damage and at the same time ameliorating such damage [19].

CCR2 protein poses 374 amino acids. The CCR2-V64I polymorphism (CCR2 G190A) is a transition mutation that substitutes valine 64 of CCR2 to isoleucine. The amino-terminal domain of CCR2 is necessary for binding of MCP1. Studies assumed that *CCR2 V64I* mutation has no impact on CCR2 expression level [12].

In this study, the *CCR2 V64I* GA + AA genotypes & A allele frequencies were significantly higher among ESRD-children when compared with healthy subjects, suggesting that the A allele of *CCR2 V64I* might be considered as an allelic variant predisposing to end stage renal disease in children with CKD and a risk marker for the ESRD development. Furthermore, the frequency of GG genotype and G allele were significantly higher among the control subjects, assuming that the wild G allele could be protective allele against the ESRD development.

Similarly, Sezgin *et al.* found that the frequency of *CCR2-V64I* mutant genotype was significantly higher than its frequency in the healthy controls and stated that, this higher mutation frequency could be related to the heaviness of the chronic renal failure and especially in the cases with chronic disease. They also claimed that CCR2 is the only one of the chemokine receptors expressed by inflammatory cells after renal injury [12].

Nephropathy as well as atherosclerosis development are thought to be consequent of chemokine signals. CCR2 & CCR5 expression by monocytes and smooth muscle cells in the vascular wall is reported to be enhanced by several cytokines or lipoproteins, suggesting that CCR-mediated signals may play a key role in the development of atherosclerosis. Nahajima *et al.*, reported that *CCR2 64I*-positive patients displayed greater mean carotid artery intima-media thickness (IMT) by B-mode ultrasonography than those without this genotype [20]. Moreover, Ana *et al.* declared that the *CCR2-V64I* variant in CCR2 is significantly associated with coronary artery calcification [21].

Recently, the CCL2/CCR2 axis attracted an increasing interest due to its negotiable association with the tumor progression and metastasis. CCL2 can be synthesized by metastatic tumour cells and stromal cells in the tumor microenvironment, with subsequent recruitment of CCR2 expressing monocytes or macrophages that promote the subsequent extravasation of tumour cells [22].

Kuper *et al.*, stated that the CCL2/CCR2 signal axis was contributory on the transmission of cell information as well as cell migration, which could upregulate cell proliferation and migration ability of renal cell carcinoma by autocrine [23].

In respect to its impact on the success and survival of renal allograft after transplantation, this study demonstrated that statistically significant differences regarding the *CCR2 V64I* genotypes frequencies between children suffering acute rejection after renal transplantation and those with successful renal transplantation. There were statistically significant differences regarding *CCR2-64I* A and G alleles frequencies, where the *CCR2-64I* A allele displayed a higher frequency among the rejection (+) group & the G allele frequency was higher among the rejection (-) group. Furthermore, the *CCR2-64I* A allele may be considered as an allelic variant and risk marker contributing to acute graft rejection in these patients.

In accordance with these results, Gorg *et al.* reported that a significant risk of acute renal transplant rejection was found in patients who possessed the *CCR2-64I* allele. They were assuming that the *CCR2* dimorphism consisting of valine/isoleucine amino acid substitution at position 64, appears to result in significant conformational changes in the structure of the protein. Therefore, the complex of MCP1 and its receptor (CCR2-64I) might promote the migration of monocytes into the transplanted kidney [4].

During acute allograft rejection, T effector cells and monocytes are attracted into the transplant producing a characteristic vascular or tubular infiltrate. This complex process of the extravasation and influx of leukocyte subsets into the site of tissue injury appears to be mediated by the expression of MCP-1 together with its corresponding CCR2 receptor which can be detected in the mononuclear cells infiltrating the kidney graft [24].

On the other hand, with contrariness with these results, Abdi et al, reported significant reductions in the risk of acute renal graft rejection in the recipients possessing the *CCR2-64I* allele [7] and supported their findings by murine model, where *CCR2* knockout mice have been shown to have decreased T cell proliferation and impaired monocyte recruitment with less interferon γ production in response to foreign antigens, leading to less inflammation [25].

Meanwhile, Kang *et al.* found no difference in the incidence of rejection among recipients stratified by the *CCR2-V64I* genotype [26].

Other conflicting results were demonstrated upon studying the influence of *CCR2-V64I* genotype on transplanted organs other than the kidney, where Simeoni *et al.* & Schroppel *et al.* reported reduced incidence of cardiac and hepatic graft rejection in patients with the *CCR2-V64I* genotype [27, 28]

Studying the genetic polymorphisms of *CCR2* is a promising field, however data on the influence of CCR2-V64I on the development and progression of ESRD as well as success and survival of renal allograft after transplantation are still controversial. More intense understanding in this field may lead to the improved life

style of these children not only at a level of disease pathogenesis and progression but also designation of novel therapeutic modalities to improve the future life of these unfortunate children.

In summary, the A allele of the *CCR2-V64I* gene polymorphism showed a significantly higher frequency among children with chronic kidney disease and assumed to be a risk marker for the end stage renal disease development. Its frequency was also significantly higher among renal transplantation recipients with acute renal graft rejection.

Conflict of interest

The authors declare that they have no competing interests.

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