Analysis of the IL-10, IL-12, and TNF-α Gene Polymorphisms in Patients With Vesicoureteral Reflux Among the Southeast Iranian Population

Dor Mohammad Kordi Tamandani, 1,7 Nasim Naemi, 2 Ali Ghasemi, 2 Taybe Baranzahi, 3 and Simin Sadeghi-Bojd 1

1 Department of Biology, University of Sistan and Baluchestan, Zahedan, IR Iran
2 Department of Biology, University of Sistan and Baluchestan, Zahedan, IR Iran
3 Children and Adolescent Health Research Center Zahedan, University of Medical Sciences, Zahedan, IR Iran

Abstract

Background: Vesicoureteral reflux (VUR) is a common childhood disorder that is characterized by the abnormal movement of urine from the bladder into the ureters or kidneys.

Objectives: The aim of this study was to determine whether the genetic polymorphisms of the IL-10, IL-12, and TNF-α genes are involved in the development of VUR.

Patients and Methods: The tetra amplification mutation refractory system-polymerase chain reaction (Tetra-ARMS PCR) was applied to analyze the four polymorphic sites of the IL-10AG-1082, IL-10CA597, IL-12CA1188, and TNF308GA genes in 124 VUR children and 110 healthy controls.

Results: A significant, highly increased risk of VUR disease was found for the CA, AA, and combined genotypes of IL-10CA597 (OR = 5.2, 95% CI: 1.80 - 18.25; P = 0.0006, OR = 9.1, 95% CI: 1.11 - 122.75; P = 0.02, OR = 5.3, 95% CI: 1.82 - 18.61; P = 0.00052, respectively); the AG, GG, and AG + GG genotypes of IL-10AG-1082 (OR = 12.8, 95% CI: 2.9 - 113.9; P = 0.00003, OR = 12.62, 95% CI: 2.93 - 114.53; P = 0.00003, respectively); and the AA genotype of IL-12 (AA, OR = 0.19, 95% CI: 0.5 - 0.55; P = 0.0006). The frequency of the CA allele in both IL-10CA and IL-12CA was greater in patients with VUR than in the healthy controls. No association was found between TNF308GA and the risk of VUR.

Conclusions: The results demonstrated significant associations between the IL-10 (AG-1089, IL-10CA) and IL-12 (AA) gene polymorphisms and a highly increased risk of VUR.

Keywords: VUR, Polymorphism, IL-10, IL-12, TNF-α

1. Background

Vesicoureteral reflux (VUR) is a heterogeneous disease in which a reverse flow of urine occurs from the bladder into the ureters and kidneys. The disease is more prevalent in newborn boys than in girls (1). VUR is generally classified as either primary or secondary reflux. Primary VUR is a congenital anomaly that occurs during embryonic growth (2). The cause of secondary VUR is an increased bladder outflow obstruction and the resultant high-pressure bladder situations (3). According to an international reflux study in children, VUR can be graded as I, II, III, IV, or V (4). The true prevalence of VUR is unknown in many populations, although it has been estimated that the prevalence of VUR is 0.4% - 1.8% in healthy children and 30% in children with urinary tract infections (UTI). Additionally, it has been reported that the prevalence of VUR is significantly higher in children whose patients have the disease (5-7). Linkage analysis has revealed some chromosomal regions that may contain the genes responsible for VUR, such as chromosomes 6p21, 10q26, and 19q13. Some families with VUR have been linked to the HLA locus on chromosome 6p21 (8, 9). The pattern of transmission of VUR can be multifactorial or autosomal-dominant inheritance with variable penetrance, autosomal recessive, and X-linked disease (10, 11). The effects of cytokine variation on the development of VUR have also been detected (12). Interleukin-10 (IL-10) is a cytokine with an anti-inflammatory activity that inhibits the production and function of TNF-α, IL-1, IL-6, IL-12, and IFN-γ, which has been located on chromosome 1 at 1q31-1q32 (13). IL-10 is a stimulatory factor for mast cells, B cells, and thymocytes, and it acts on many other cell types, including monocytes/macrophages, T cells, NK cells, neutrophils, endothelial cells, and PBM. It has been shown that several diseases are associated with the polymorphism of the IL-10 promoter region, and high IL-10 production is...
associated with autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (14). IL-12 is a pro-inflammatory cytokine that stimulates the production of IFN-γ. IL-12 produces dendritic cells and phagocytes in response to pathogens during infection. It is involved in the differentiation of naïve T cells into Th1 cells (15). Tumor necrosis factor α (TNF-α) is a cytokine that is involved in systemic inflammation. TNF-α is produced by activated macrophages, lymphocytes, and T helper cells, and it stimulates the synthesis of other growth factors and cytokines. Its gene is located on chromosome 6p21.3 (16). TNF-α is also produced by intrinsic kidney cells. Direct cytotoxicity occurs from this cytokine to the renal cells, which leads to direct renal injury, necrotic cell death, and apoptosis. It can also cause alterations of the intra glomerular blood flow and a decrease of glomerular filtration as a result of the disequilibrium between the factors promoting vasoconstriction and vasodilatation in order to change the function of endothelial cells (17).

2. Objectives
The purpose of this study was to assess the role of polymorphic variants of the IL-10AG-1082, IL-10CA597, IL-12CA1188, and TNF308GA genes in the development of VUR in samples of the Iranian population.

3. Patients and Methods

3.1. Subjects and Clinical Data
The study was conducted from September 2010 to September 2011 in Zahedan, Sistan and Baluchestan province, southeast Iran. The study involved 124 VUR patients (73 females and 51 males) with different grades of progression (I = 5, II = 19, III = 40, IV = 14, and V = 6). The VUR patients had a mean age of 2.51 years (± 2.89) and a mean weight of 11.15 kg (± 6). The study also involved 110 healthy controls (66 females and 44 males) with a mean age of 2.79 years (± 5.9) and a mean weight of 11.23 kg (± 5.9) (Table 1).

3.2. DNA Extraction and PCR
Blood samples were collected from all participants via venipuncture and then kept in EDTA-coated tubes to be used for DNA extraction at the time of clinical examination. The control samples were obtained from individuals without any history of renal disease or inflammation disturbance. Single nucleotide polymorphisms (SNPs) located in and around the IL-10, IL-12, and TNF-α genes were detected by PCR using the tetra amplification refractory mutation system (ARMS) designed for the detection of various alleles and genotypes. Two outer and two inner primers (forward and reverse) were used to detect variations in the IL-10, IL-12, and TNF-α genes, as previously reported (18).

3.3. Statistical Analyses
SPSS version 10.0 (SPSS, Chicago, IL) and epical info version 7 were used for all statistical analyses. The associations of polymorphisms in the IL-10AG-1082, IL-10CA597, IL-12CA1188, and TNF308GA genes with the risk of developing VUR were identified by estimating the odds ratios (OR) and 95% confidence intervals (95% CL) using epical info version 7. A significant P value was held to be less than 0.05. Pearson’s χ² test was used to analyze the categorical variables.

4. Results
As presented in Table 2, the CA, AA, and combined CA + AA genotypes of IL-10 (CA) significantly increased the risk of VUR (OR = 5.2, 95% CI: 1.80 - 18.25; P = 0.0006; OR = 5.3, 95% CI: 1.82 - 18.61; P = 0.00052, respectively). All of the genotype forms (AG, GG, and AG + GG) of IL-10AG highly increased the risk of the disease (OR = 12.6, 95% CI: 2.9 - 113.9; P = 0.00003; OR = 15, 95% CI: 0.6 - 117.31; P = 0.06; OR = 12.62, 95% CI: 2.93 - 114.53; P = 0.00003, respectively).

The allele frequency for the C of the IL-10CA597 and IL-12CA1188 genes showed statistically significant differences between the VUR cases and the controls (P = 0.001 and P = 0.025, respectively) (Table 3).

As shown in Table 4, the gene-gene interaction analysis of IL-12CA and IL-10AG showed a significant correlation between the CC/AG (OR = 9.43, 95% CI: 1.15 - 438.76; P = 0.022) and CA/AG (OR = 8.47, 95% CI: 0.89 - 417.57; P = 0.034) genotypes and the risk of developing VUR. This analysis also showed significance for IL-12CA/IL-10CA in terms of the CC/AA and CA/AA genotypes (P = 0.000015 and P = 0.024, respectively). In addition, it was observed that the gene-gene interaction of IL-10AG and IL-10CA (AG/CA and AG/AA) was significantly associated with an increased risk of VUR (P = 0.0020 and P = 0.03, respectively).
Table 1. Demographic Characteristics of the Patients With VUR and the Healthy Controlsa

| Variation | VUR Cases | Controls |
|-----------|-----------|----------|
| Sex       |           |          |
| Male      | 51        | 44       |
| Female    | 73        | 66       |
| Weight, kg| 11.5 ± 6  | 11.23 ± 5.9 |
| Age, y    | 2.51 ± 2.89 | 2.79 ± 5.9 |
| Grade     |           |          |
| I         | 5         | 5        |
| II        | 19        | 19       |
| III       | 40        | 40       |
| IV        | 14        | 14       |
| V         | 6         | 6        |

aValues are expressed as No. or mean ± SD.

Table 2. Number and Frequency of the IL10AG-1082, IL10CA597, IL12CA1188, and TNF308GA Genotypes in VUR Patients and Healthy Controlsa

| SNPs      | VUR Cases, n = 124 | Controls, n = 110 | OR       | CI         | P Value    |
|-----------|--------------------|-------------------|----------|------------|------------|
| IL10CA597 |                    |                   |          |            |            |
| CC        | 5 (4.03)           | 20 (18.18)        | Reference|            |            |
| CA        | 114 (91.93)        | 88 (80)           | 5.2      | 1.80 - 18.25 | 0.0006     |
| AA        | 5 (4.03)           | 2 (1.82)          | 9.1      | 1.11 - 122.75 | 0.02       |
| CA + AA   | 119 (95.97)        | 90 (81.82)        | 5.3      | 1.82 - 18.61 | 0.00052    |
| IL10AG-1082|                   |                   |          |            |            |
| AA        | 2 (1.61)           | 19 (17.27)        | Reference|            |            |
| AG        | 120 (96.77)        | 90 (81.82)        | 12.6     | 2.9 - 113.9 | 0.00003    |
| GG        | 2 (1.61)           | 1 (0.91)          | 15       | 0.6 - 1176.31 | 0.06       |
| AG + GG   | 122 (98.39)        | 91 (82.73)        | 12.62    | AG + GG    | 0.00003    |
| IL12CA1188|                   |                   |          |            |            |
| CC        | 65 (52.42)         | 53 (48.18)        | Reference|            |            |
| CA        | 54 (43.55)         | 35 (31.82)        | 1.26     | 0.69 - 2.29 | 0.48       |
| CA + AA   | 59 (47.58)         | 57 (51.82)        | 0.84     | 0.49 - 1.46 | 0.6        |
| TNF308GA  |                   |                   |          |            |            |
| GG        | 115 (92.74)        | 105 (95.45)       | Reference|            |            |
| GA        | 8 (6.45)           | 5 (4.55)          | 1.46     | 0.41 - 5.8  | 0.58       |
| GA + AA   | 9 (7.26)           | 5 (4.55)          | 1.64     | 0.48 - 6.44 | 0.42       |

aValues are expressed as No. (%).

Table 3. Allele Frequency (%) Among Individual VUR Cases and Healthy Controlsa

| Genes     | VUR Cases | Controls | P Value |
|-----------|-----------|----------|---------|
| IL10CA597 |           |          |         |
| C         | 124 (50)  | 128 (58.18) | 0.001   |
| A         | 124 (50)  | 92 (41.82)  |         |
| IL10AG-1082|          |           |         |
| A         | 124 (50)  | 128 (58.18) | 0.09    |
| G         | 124 (50)  | 91 (41.36)  |         |
| IL12CA    |           |          |         |
| C         | 184 (74.19) | 141 (64.09) |         |
| A         | 64 (25.81) | 79 (35.91)  | 0.025   |
| TNF308GA  |           |          |         |
| G         | 238 (95.97) | 215 (97.73) |         |
| A         | 10 (4.03)  | 5 (2.27)    | 0.306   |

aValues are expressed as No. (%).
Table 4. Gene Combinations of the IL12CA, IL10AG, IL-10 AG, IL-10 AC, and TNF308GA Genotypes in the VUR Cases and Healthy Controls

| Genes          | VUR Cases | Controls | OR      | CI       | P Value |
|----------------|-----------|----------|---------|----------|---------|
| IL12CA/IL10AG  | CC/AA     | 1        | 7       | Reference|
|                | CC/AG     | 63       | 46      | 9.43     | 1.15 - 438.76 | 0.022    |
|                | CA/AA     | 1        | 5       | Reference|
|                | CA/AG     | 52       | 30      | 8.47     | 0.89 - 417.57 | 0.034    |
| IL12CA/IL10CA  | CC/CC     | 2        | 7       | Reference|
|                | CC/CA     | 6        | 45      | 0.47     | 0.064 - 5.71  | 0.6      |
|                | CC/AA     | 30       | 1       | 78.55    | 6.43 - 4870.85 | 0.000015 |
|                | CA/CC     | 3        | 8       | Reference|
|                | CA/CA     | 49       | 27      | 4.75     | 1.03 - 30.09  | 0.000015 |
| IL12CA/TNF308GA| AG/GG     | 113      | 86      | Reference|
|                | AG/GA     | 7        | 4       | 1.33     | 0.33 - 6.40   | 0.76     |
| IL10AG/TNF308GA| CA/GG     | 105      | 85      | Reference|
|                | CA/GA     | 8        | 3       | 2.35     | 0.50 - 12.97  | 0.35     |

5. Discussion

Anti-inflammatory and pro-inflammatory cytokines play an important role in the regulation of the immune system in response to various microorganisms (19). It has been confirmed that IL-10 genes increase the risk of UTIs. TNF-α, IL-1, and IL-6 synthesized by renal cells performing in an autocrine and paracrine styles may provoke a variety of effects on different renal structures, and they play a major role in the expansion and progression of some renal disorders. The renal effects of inflammatory cytokines are linked to the expression of different molecules, alteration of the extracellular matrix, intra glomerular hemodynamic abnormalities, glomerular basement membranes, necrosis, apoptosis, oxidative stress, and endothelial permeability (17). In this study, a significant association was found between the genotypic frequency of IL-10CA and IL-10AG and an increased risk of VUR. Fidan et al. (20) reported that the IL-10 gene polymorphisms are linked to the development of reflux nephropathy in patients with primary VUR, and it was also noted that the GCC/GCC and ACC/ACC haplotypes for the three IL-10 promoter loci were associated with an increased risk of renal scarring. Finally, they pointed out that certain genotypes of cytokines’ gene polymorphisms may be associated with increased or decreased susceptibility to reflux nephropathy, with varying trends seen for patients with high-grade and low-grade VUR. Manchanda et al. (21, 22) demonstrated that the AA genotype of IL-10-1082 G/A and both variants of TNF-α (− 308 and + 488) have a significant association with an increased susceptibility to end-stage renal disease. Bantis et al. (23, 24) showed that the IL-10 gene’s G-1082A polymorphism plays an important role in the development of nephropathy and focal segmental glomerulosclerosis (FSGS). Some studies have reported that the TNF-α AA genotype is not associated with reflux nephropathy and renal scarring, although the TNF-α-308A allele could be connected to a higher susceptibility to VUR (25, 26). In addition, Bienias et al. (27) demonstrated that there were no significant differences between children with unilateral and bilateral vesicoureteral reflux in terms of the serum level of TNF-α. Shu et al. (28) showed the effect of TNF-α polymorphism on the susceptibility to IgA nephropathy. Pro-inflammatory cytokines such as TNF-α initiate the parenchymal damage that leads to renal scarring. Other studies have shown increased levels of this cytokine (TNF-α) in embryonic dysplastic kidneys (29).

5.1. Conclusion

The results of the present study demonstrated significant associations between IL-10 (AG-1089, IL-10CA) and IL-12
Kordi Tamandani DM et al.

(AA) gene polymorphism and a highly increased risk of VUR. Ultimately, more studies involving a large sample size in various genetic populations are recommended for the verification of the present data and in order to elucidate the results.

Acknowledgments

We would like to thank the department of biology, University of Sistan and Baluchestan, Zahedan, Iran, and the department of pediatric nephrology, children and adolescents health center, Zahedan University of Medical Sciences, for providing financial support for this project.

References

1. Gargollo PC, Diamond DA. Therapy insight: What nephrologists need to know about primary vesicoureteral reflux. Nat Clin Pract Nephrol. 2007;3(10):534-6. doi: 10.1038/ncpnep0860. [PubMed: 17895932]
2. Sharifian M, Boroujerdi HZ, Dalirani R, Maham S, Sepahi MA, Karimi A, et al. Spontaneous resolution of vesicoureteral reflux (VUR) in Iranian children: A single center experience in 533 cases. Nephrourol Mon. 2011;3:1-5.
3. Oksuz M, Genc G, Ozkaya O, Bek K, Arslan S, Sariyaka S. Clinical Characteristics of Urinary Tract Infections in A Tertiary Center. Yeni Tip Dergisi. 2012;30(1):56.
4. Lebowitz RL, Olbing H, Parkkulainen KV, Smellie JM, Tamminen-Mobius TE. International system of radiographic grading of vesicoureteral reflux. International Reflux Study in Children. Pediatr Radiol. 1985;15(2):105-9. [PubMed: 3957102]
5. Williams G, Fletcher JT, Alexander SI, Craig JC. Vesicoureteral reflux. J Am Soc Nephrol. 2000;11(5):847-62. doi: 10.1681/asn.20000730. [PubMed: 10916103]
6. Sugn J, Skog S. Surgical management of vesicoureteral reflux in children. Pediatr Nephrol. 2012;27(4):551-61. doi: 10.1007/s00467-013-2519-2. [PubMed: 22695451]
7. Tegkul S, Reddmiller H, Hoebbeke P, Kovcara R, Nijman RJ, Radmayr C, et al. EAU guidelines on vesicoureteral reflux in children. Eur Urol. 2012;62(3):534-42. doi: 10.1016/j.eururo.2012.05.059. [PubMed: 22689873]
8. Sanna-Cherchi S, Reeve A, Hensle T, Caridi G, Izi C, Kim YY, et al. Familial vesicoureteral reflux: testing replication of linkage in seven new multigenerational kindreds. J Am Soc Nephrol. 2005;16(6):1781-7. doi: 10.1681/ASN.200412034. [PubMed: 15829711]
9. van Eerde AM, Duran K, van Riel E, de Kovel CG, Koelman BP, Knoers NV, et al. Familial vesicoureteral reflux and obstructive nephropathy. J Pediatr Urol. 2009;5(4 Pt 1):131-7. doi: 10.1016/j.pjpurol.2009.04.014. [PubMed: 19344880]
10. Puri P, Gosemann JH, Darlow J, Barton DE. Genetics of vesicoureteral reflux. Nat Rev Urol. 2013;10(5):359-52. doi: 10.1038/nrurol.2013.115. [PubMed: 23689976]
11. Fletcher J, McDonald S, Alexander SI, New Zealand Pediatric Nephrology A. Australian. Prevalence of genetic renal disease in children. Pediatr Nephrol. 2013;28(2):251-6. doi: 10.1007/s00467-012-2306-6. [PubMed: 23052649]
12. Kordi-Tamandani DM, Sadeghi-Bojd S, Torkamanzehi A, II-I9 and II-20 genes polymorphisms and haplotype analysis in a vesicoureteral reflux population. Hum Immunol. 2013;74(1):331-4. doi: 10.1016/j.humimm.2012.09.005. [PubMed: 23000500]
13. Commins S, Steinkv J, Borish I. The extended II-10 superfamily: II-9, II-19, II-20, II-22, II-24, II-26, II-28, and II-29. J Allergy Clin Immunol. 2008;121(5):1008-11. doi: 10.1016/j.jaci.2008.02.026. [PubMed: 18409598]
14. Liao TC, Liang WG, Chen FW, Hsu JH, Yang JJ, Chang MS. IL-19 induces production of IL-6 and TNF-alpha and results in cell apoptosis through TNF-alpha. J Immunol. 2002;169(8):4288-97. [PubMed: 12370160]
15. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol. 2003;3(2):331-46. doi: 10.1038/nri1001. [PubMed: 12563297]
16. Horiuchi T, Mitoma H, Harashima S, Tuskamoto H, Shimoda T. Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents. Rheumatology (Oxford). 2010;49(1):125-28. doi: 10.1093/rheumatology/keq031. [PubMed: 20194223]
17. Donate-Correa J, Martin-Nunez E, Muros-de-Fuentes M, Morales-Ramirez A, Barrientos-Navarro Gonzalez JF. Inflammatory cytokines in diabetic nephropathy. J Diabetes Res. 2015;2015:948447. doi: 10.1155/2015/948447. [PubMed: 25785280]
18. Koks S, Kungs K, Ratsep R, Karelson M, Silm H, Varas E. Combined haplotype analysis of the interleukin-19 and -20 genes: relationship to plaque-type atherosclerosis. Genes Immun. 2004;5(3):662-7. doi: 10.1038/sj.gene.6364144. [PubMed: 15495954]
19. Rollino C, D’Urso L, Beltrame G, Ferro M, Quattrociocchio G, Quarello F. Vesicoureteral reflux in adults. G Ital Nefrol. 2011;28(6):599-611. [PubMed: 22676071]
20. Fidan K, Gonen S, Soylenzoglu O. The association of cytokine gene polymorphism with reflux nephropathy. J Pediatr Urol. 2013;9(5):553-8. doi: 10.1016/j.pjpurol.2012.07.017. [PubMed: 23905885]
21. Manchanda PK, Kumar A, Kaul A, Mittal RD. Correlation between a gene polymorphism of tumor necrosis factor-alpha (G/A) and end-stage renal disease: a pilot study from north India. Clin Chim Acta. 2006;370(2-4):352-7. doi: 10.1016/j.cca.2006.02.002. [PubMed: 16545788]
22. Manchanda PK, Singh RK, Mittal RD. Cytokine (IL-10 -1082 and -819) gene polymorphism in reflux nephropathy. J Nephrol. 2009;22(5):477-81. doi: 10.1007/s11529-008-0822-z. [PubMed: 19196047]
23. Bantis C, Heering PJ, Akser S, Klein-Vehne N, Grabensee B, Ivens K. Association of interleukin-10 gene G-1082A polymorphism with the progression of primary glomerulonephritis. Kidney Int. 2004;66(2):288-94. doi: 10.1111/j.1523-1755.2004.00703.x. [PubMed: 15204036]
24. Bantis C, Heering PJ, Akser S, Schwaedt C, Grabensee B, Ivens K. Influence of interleukin-10 gene G-1082A polymorphism on recurrent IgA nephropathy. J Nephrol. 2008;21(3):69-41. doi: 10.1007/s11529-007-0164-6. [PubMed: 19348880]
25. Solari V, Ennis S, Caccio S, Puri P. Tumor necrosis factor-alpha gene polymorphism in reflux nephropathy. J Urol. 2004;172(4 Pt 2):1604-6. [PubMed: 15377710]
26. Pardo R, Malaga S, Alvarez V, Coto E. Vesicoureteric reflux and tumor necrosis factor-alpha gene polymorphism. J Pediatr Urol. 2007;3(2):24-7. doi: 10.1016/j.pjpurol.2006.01.003. [PubMed: 18497693]
27. Bienias S. Serum TGF-β and TNF concentrations in children with reflux and obstructive nephropathy. Int Rev Allergol Clin Immunol. 2010;18(1):31-8.
28. Shu KH, Lee SH, Cheng CH, Wu MJ, Lian JD. Impact of interleukin-1 receptor antagonist and tumor necrosis factor-alpha gene polymorphism on IgA nephropathy. Kidney Int. 2000;58(2):783-9. doi: 10.1046/j.1523-1755.2000.00227.x. [PubMed: 10916103]
29. Cale CM, Klein NJ, Winyard PJ, Woolf AS. Inflammatory mediators in human renal dysplasia. Nephrol Dial Transplant. 2000;15(2):473-83. [PubMed: 10648662]