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Association of *Interleukin-31* gene polymorphisms with risk of cryptorchidism in a Chinese population

Bing Zou, MD[a,b], Zhihai Yu, MD[c], Jing Huang, MD[a,b,*], Chunlin Tan, MD[a,b], Haiyun Wang, MD[a,b], Jian Fu, MD[a,b], Xin Li, MD[a,b], Xiaojun Wang, MD[a,b], Shu Cui, MD, PhD[a,b], Tielong Tang, MD, PhD[a,b,*]

Abstract

This study aims to investigate the possible association between Interleukin-31 (IL-31) gene polymorphisms and cryptorchidism risk.

Two single nucleotide polymorphisms of *IL*-31, rs7977932 (C/G) and rs4758680 (C/A), were selected to be investigated in this study. Polymerase chain reaction-restriction fragment length polymorphism methods were used to discriminate the selected single nucleotide polymorphisms of *IL*-31 gene. A hospital-based case-control study of 112 cryptorchidism patients and 425 healthy controls was conducted.

The frequencies of the C allele of rs4758680 in the patients with cryptorchidism were significantly higher compared with those in controls (89% vs 83%, $P=.02$, OR=0.58, 95% CI=0. 37–0.92). Compared with CC genotype in dominant model, notable decreased frequencies of A carriers (CA/AA genotypes) were observed in cryptorchidism patients ($P=. 03$, OR=0.58, 95% CI= 0.35–0.96).

Results demonstrated that *IL*-31 gene polymorphisms were associated with the genetic susceptibility to cryptorchidism in a Chinese population. Compared with CC genotype, the A carriers (CA/AA genotypes) of rs4758680 were protect factors in cryptorchidism susceptibility.

Abbreviations: IL-31 = interleukin-31, IL-31RA = interleukin-31 receptor A, IL-6 = interleukin-6, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SNPs = single nucleotide polymorphisms.

Keywords: association, cryptorchidism, *IL-31*, polymorphisms

1. Introduction

Cryptorchidism, defined as a failure of the descent of 1 or 2 testicle(s) and its associated structures into the scrotum, has been recognized as one of the most common congenital anomalies in newborn boys.[1] Approximately one-third of the premature boys are affected by cryptorchidism, and about 2% to 8% of full-term boys have at least 1 undescended testicle; moreover, the prevalence of cryptorchidism in some countries has increased.[2]-

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[a] Department of Urology, [b] Urogenital Diseases Lab, Affiliated Hospital of North Sichuan Medical College, Nanchong, [c] Department of Urology, Chongqing Three Gorges Central Hospital, Wanzhou, People's Republic of China.

[*] Correspondence: Tielong Tang, Affiliated Hospital of North Sichuan Medical College, Nanchong, 637000, Sichuan, People's Republic of China (e-mail: cdzt2004@163.com); Jing Huang, Department of Urology, Affiliated Hospital of North Sichuan Medical College, Nanchong, 637000, Sichuan, People's Republic of China (e-mail: jinghuang129@sina.com).

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However, the prevention of this disease is a significant challenge for clinicians and researchers. Although cryptorchidism is often considered a mild malformation and the affected individuals do not experience uncomfortable symptoms in the short term, they have the potential to develop sub/infertility, testicular neoplasm, and other bothering complications in the long run.[2,3] The multifactorial etiological and risk factors for cryptorchidism have been partially illustrated, but congenital cryptorchidism itself always occurs as an isolated disorder with no obvious reason.[4] Although the degrees of maldescent are variable, the testicles of some gene knockout mice incompletely descend along the pathway from their initial high position in the abdomen to the bottom of the scrotum.[5] Scholars have detected familial clustering and increased recurrence risk ratios in third-degree to first-degree relatives of patients with cryptorchidism.[1] Hence, genetic factors may contribute to the development of cryptorchidism.

Interleukin-31 (IL-31) is a newly discovered member of the GP130/IL-6 cytokine family, which mainly includes interleukin-11, interleukin-27, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotrophin-1 in possession of the shared receptor subunit; IL-31 is produced by CD4+ T cells, particularly those skewed toward the T-helper 2 type cytokine profile, after being activated.[6–9] IL-31 signals through a heterodimeric functional receptor complex composed of IL-31 receptor A subunit and oncostatin M-specific receptor β subunit; the former belongs to the gp130-subfamily of type 2 cytokine receptors, and the latter activate the Janus kinase-signal transducer and activator of transcription and mitogen activated protein kinase signaling pathways.[6–9] Emerging experimental evidence suggests the involvement of IL-31 in many fundamental

physical processes, such as development and growth of neurons, myocardium, immune system, reproductive system, respiratory system, and bone metabolism. Researchers have detected relatively high genetic expression of mRNA encoding *IL-31* copresent with *IL-31 receptor A* and *oncostain M* in a variety of tissue cells.[10] Initially described as a pro-inflammatory cytokine that promotes T-helper 2 response in multiple immune function, IL-31 could significantly induce the release of interleukin-6 (IL-6), whose serum expression level is positively related to increased cryptorchidism risk.[11,12] A previous case-control study interviewed the mothers of affected (83 cryptorchidism cases) and unaffected boys (129 controls) and reported 9.6% proportion of at least 1 episode of asthma in cases versus 1.6% in controls, and results demonstrated that boys born with cryptorchidism were more likely to present with asthma and partially indicated the involvement of IL-31 in asthma.[13,14] Thus, IL-31 may influence the development of cryptorchidism.

IL-31 is involved in cutaneous pathologies, inflammatory bowel diseases, atopic dermatitis, respiratory inflammation, and some types of tumors;[15] however, the involvement of IL-31 in cryptorchidism has not been reported yet. We assume that cryptorchidism risk may associate with *IL-31* gene single nucleotide polymorphisms (SNPs). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods are economical and effective techniques used to detect SNPs and genetic mutations. In the present study, we elucidated the role of *IL-31* gene polymorphisms, namely, rs7977932 (C/G) and rs4758680 (C/A), in patients with cryptorchidism in a Chinese population.

2. Materials and methods

2.1. Study subjects

A hospital-based case-control study of 112 unrelated patients with cryptorchidism (only isolated cryptorchidism cases) and 425 control subjects was conducted. The patients who participated in the study were recruited from the Affiliated Hospital of North Sichuan Medical College between January 2010 and December 2015. The patients underwent physical examination shortly after birth and were examined once again at 3 months of age to determine their abnormality. Premature or low-birth-weight infants and subjects with any personal or family history of cryptorchidism, serious diseases, or systemic abnormalities were excluded from the study. The examination technique and the definition of cryptorchidism developed by Scorer[16] were applied. All of the examinations were performed with the child in supine position under warm condition. Testicular position was recorded after the manipulation of the testis to the most distal position along the pathway of normal descent by using firm but not forced traction. The diagnosis was reconfirmed before orchidopexy and when the patients are around 1-year old. A total of 97 cases manifested unilateral cryptorchidism, and 15 cases presented with bilateral cryptorchidism. In bilateral cases, the testis position was decided by the higher one. The testes of 32, 27, 24, 19, and 10 patients were located in the superficial inguinal pouch, prescrotal region, external ring, inguinal canal, and internal ring, respectively. A group of control subjects (mean age 5.8 ± 1.3 years, 1 month–15 years) were presenting for resection for foreskin. All the subjects belong to Han Chinese population living in Sichuan province of southwest China. This study was approved by the ethics committee of the Affiliated Hospital of North Sichuan Medical College. The parents of all the participants were given written informed consent to participate in this study.

2.2. DNA extraction and genotyping

Two SNPs of *IL-31* gene, rs7977932 (C/G), and rs4758680 (C/A), were genotyped. Genomic DNA of each individual was extracted from 200 μL of EDTA-anticoagulated peripheral blood samples by using a DNA isolation kit from Bioteke (Peking, China). The procedure was performed according to the manufacturer's instructions. Genotyping of the 2 selected SNPs was performed through Polymerase chain reaction-restriction fragment length polymorphism methods. Primers were designed using software Primer 3 (http://bioinfo.ut.ee/primer3–4.0/primer3/) as shown in Table 1.

DNA fragments containing the polymorphisms were amplified in a total volume of 25 μL, including 2.5 μL of $10 \times$ PCR buffer, 1.5 mmol L^{-1} $MgCl_2$, 0.15 mmol L^{-1} dNTPs, 0.5 mmol L^{-1} of each primer, 100 ng of genomic DNA, and 1 U Taq DNA polymerase. The PCR condition was as follows: 94°C for 4 minutes; followed by 32 cycles of 30 seconds at 94°C, 30 seconds at 64°C, 30 seconds at 72°C for rs7977932, and 30 seconds at 94°C for rs4758680, and 30 seconds at 66°C, and 30 seconds at 72°C; with a final elongation at 72°C for 10 minutes. The PCR products were digested overnight with specific restriction enzymes (Table 1). The digested products were separated using a 6% polyacrylamide gel and stained with 1.5 g/L argent nitrate.

The genotypes were confirmed by DNA sequencing analysis. About 15% of the samples were randomly selected to perform repeated assays, and the results were 100% concordant.

2.3. Statistical analysis

SPSS 22.0 (SPSS Inc, Chicago, IL) was applied to analyze all of the experimental data. The genotype frequencies of the 2 selected SNPs were obtained through directed computing. The Hardy–Weinberg equilibrium was evaluated through χ^2 test. Odds ratio (OR) and corresponding 95% confidence intervals (CI) were reported to evaluate the effects of any difference between alleles and genotypes. Probability values of 0.05 or

Table 1

Information about polymerase chain reaction-restriction fragment length polymorphism in cryptorchidism patients and control groups.

SNPs	Primer sequence (5'-3')	Annealing temperature (°C)	Allele (bp)	Enzyme	Product (bp)
rs4758680	F: gatcacccggactcaaaacgtg	60	A (263)	MboII	263
	R: ttgtgcaaaccacacctcttcg		C (210 + 53)		
rs7977932	F: ggtcagtgttgggtttgcaatg	60	G (74 + 57)	ScrFI	131
	R: ttggtgatggcacagcctcata		C (131)		

Table 2

Allele frequencies of tag SNPs in *interleukin-31* gene among patients and controls and their association with cryptorchidism risk.

SNP	Allele	Patients N = 112 (%)	Controls N = 425 (%)	OR (95% CI)	*P*
rs7977932	C	210 (94%)	768 (90%)	0.62 (0.35–1.12)	.11
	G	14 (6%)	82 (10%)		
rs4758680	C	199 (89%)	702 (83%)	**0.58 (0.37–0.92)**	**.02**
	A	25 (11%)	148 (17%)		

Note. N corresponds to the number of individuals; values in bold indicate a significant difference at the 5% level.

less than 1 ($P \leq .05$) indicated statistically significant difference between patients with cryptorchidism and controls. All statistical tests were 2 sided. Genotypic association tests in a case-control pattern assuming codominant, dominant, recessive, or overdominant genetic models were performed using SNPstats (https://www.snpstats.net/snpstats/).

3. Results

Both of the selected SNPs (rs7977932 and rs4758680) were successfully genotyped in 112 patients with cryptorchidism and 425 healthy control subjects. The genotype distributions of these polymorphisms in the control subjects were consistent with the Hardy–Weinberg equilibrium ($\chi^2 = 1.44$, $P > .05$ for rs4758680; $\chi^2 = 0.62$, $P > .05$ for rs7977932). The allele frequencies of the 2 SNPs in patients with cryptorchidism and control subjects are shown in Table 2. No significant differences were observed in the allele frequencies of rs7977932 between the patients and controls. However, the frequencies of C allele of rs4758680 in patients with cryptorchidism significantly increased compared with those in the controls (89% vs 83%). By contrast, the A allele frequencies of rs4758680 decreased (11% vs 17%) in the case group. A significantly decreased cryptorchidism risk was found to be associated with the A allele of the rs4758680 locus ($P = .02$, OR = 0.58, 95% CI = 0.37–0.92).

Analysis of the rs7977932 gene polymorphism indicated no significant difference in every single genetic model between patients with cryptorchidism and controls (Table 3).

A significantly decreased cryptorchidism risk was found to be associated with C/A-A/A genotype compared with the C/C genotype of rs4758680 polymorphisms ($P = .03$, OR = 0.58, 95% CI = 0.35–0.96) in the dominant model (Table 4).

4. Discussion

Cryptorchidism is a congenital disease that has complicated etiology affected by genetic factors, environmental factors, and disorder of hormonal actions. Accumulating lines of experimental evidence suggest that inflammatory cytokines and their accessory oxidative stress may play a role in the development of cryptorchidism. Imamoğlu et al[12] determined that patients with cryptorchidism, especially those with bilateral type, experienced high levels of oxidative stress exposure and cytokine response. Association studies reported that SNPs in some inflammation-related genes, such as *interleukin-27* gene and *interleukin-21* gene, could significantly increase the risk of cryptorchidism.[4,17] Inflammation or an inflammatory-like condition with its associated oxidative stress may participate in increased testicular temperature and male infertility, which are involved in cryptorchidism.[12,18]

Potashnik et al[19]revealed that the secretion of IL-6 is accompanied with abnormal quantities of testosterone under in vitro condition, whose biological effects on the regulation of testicular descent have been intensively investigated. Interestingly, IL-31, which is as a newly discovered inflammatory cytokine, could effectively induce the release of proinflammatory mediators, such as IL-6, in various cell types.[20] The human *IL-31* gene, which is located on chromosome 12q24.13, could encode a protein consisting of 164 amino acids.[21] IL-31 may act as a pleiotropic cytokine that exerts multiple biological effects, including hematopoietic regulation, immunity, cell proliferation, and cytokine and chemokine induction; moreover, overexpres-

Table 3

Genotype frequencies of tag SNPs in *interleukin-31* gene among patients and controls and their association with cryptorchidism.

		Patients	Controls	Logistic regression	
Genetic model	Genotype	N = 112 (%)	N = 425 (%)	OR (95% CI)	*P*
rs7977932					
Codominant	C/C	99 (88.4%)	348 (81.9%)	1.00	
	C/G	12 (10.7%)	72 (16.8%)	0.57 (0.30–1.09)	.23
	G/G	1 (0.9%)	5 (1.2%)	0.68 (0.08–5.91)	
Dominant	C/C	99 (88.4%)	348 (81.9%)	1.00	
	C/G-G/G	13 (11.6%)	77 (18.1%)	0.58 (0.31–1.08)	.09
Recessive	C/C-C/G	111 (99.1%)	420 (98.8%)	1.00	
	G/G	1 (0.9%)	5 (1.2%)	0.74 (0.09–6.37)	.79
Overdominant	C/C-G/G	100 (89.3%)	353 (83.1%)	1.00	
	C/G	12 (10.7%)	72 (16.9%)	0.57 (0.30–1.09)	.09

Note. N corresponds to the number of individuals.

Table 4

Genotype frequencies of tag SNPs in *interleukin-31* gene among patients and controls and their association with cryptorchidism.

		Patients	Controls	Logistic regression	
Genetic model	**Genotype**	**N = 112 (%)**	**N = 425 (%)**	**OR (95% CI)**	***P***
rs4758680					
Codominant	C/C	89 (79.5%)	294 (69.2%)	1.00	
	C/A	21 (18.8%)	114 (26.8%)	0.61 (0.36–1.03)	.08
	A/A	2 (1.8%)	17 (4%)	0.39 (0.09–1.7)	
Dominant	C/C	89 (79.5%)	294 (69.2%)	1.00	
	C/A-A/A	23 (20.5%)	131 (30.8%)	**0.58 (0.35–0.96)**	**.03**
Recessive	C/C-C/A	110 (98.2%)	408 (96%)	1.00	
	A/A	2 (1.8%)	17 (4%)	0.44 (0.10–1.92)	.22
Overdominant	C/C-A/A	91 (81.2%)	311 (73.2%)	1.00	
	C/A	21 (18.8%)	114 (26.8%)	0.63 (0.37–1.06)	.07

Note. N corresponds to the number of individuals. Values in bold indicate a significant difference at the 5% level.

sion of *IL-31*gene could cause dermatitis, airway hypersensitivity, and inflammatory bowel disease.[10] In addition to its function as a pro-inflammatory cytokine, IL-31 acts as an anti-inflammatory cytokine for IL-31/IL-31R interaction and as a novel negative regulator of type 2 inflammation in lungs.[22] The expression profiles of *IL-31* gene mRNA, *IL-31 RA* gene, and *OSMR* gene mRNA were detected using quantitative real-time PCR. IL-31 could induce Janus kinase-signal transducer and activator of transcription receptor activation (from 2–11-folds over basal amounts) in the cell line expressing *IL-31 RA* and *OSMR* gene.[23,24] Thus, tissues in the testis could be potential targets and may be responsive to IL-31. IL-31 could possibly affect the release of IL-6 and consequently influence the development of cryptorchidism. The present study focused on elucidating the correlation of *IL-31* gene polymorphism to cryptorchidism risk.

This retrospective study presented the first evidence of the link between cryptorchidism and *IL-31* gene polymorphisms in a Chinese Han population. We analyzed the allele and genotype frequencies of 2 SNPs, rs7977932 and rs4758680, of the *IL-31* gene in the chromosome 12 of 112 patients and 425 healthy controls in several genetic models. SNP rs4758680 of *IL-31* gene was associated with the genetic susceptibility of cryptorchidism in the selected Chinese population. The A carriers (CA/AA genotypes) were associated with a 42% decreased cryptorchidism risk in the dominant model and were protect factors in cryptorchidism susceptibility. The selected SNPs in the *IL-31* gene are both located in the intron region. For rs4758680, statistical significance was observed between patients and healthy controls in the dominant genetic model. Although located in the noncoding region, SNP rs4758680 may influence the conduct of other genes rather than the function or structure of IL-31 by alerting the coding of amino acids in the *IL-31* gene to be susceptible to cryptorchidism. The data described in our present study are consistent with those described in studies focusing on association between *IL-31* gene polymorphisms an dilated cardiomyopathy and systemic lupus erythematosus.[21,25] Increasing lines of evidence indicate that introns, which are located in noncoding regions, could initiate and enhance gene expression in a wide range of biological population by using the proposed mechanism, called intron-mediated enhancement.[26] Zhou et al[27] suggested that introns may have evolved to function as network controlling molecules in higher organisms and could free them from the constraints of a simple single-output protein-based genetic operating system. Although no association was found between rs7977932 polymorphisms and cryptorchidism, this observation may have resulted from the limited samples in our study. Thus, further evaluation should be conducted in a larger sample size. Our results provide evidence for the association between the selected inflammation-related gene polymorphisms and cryptorchidism.

Cytokines, such as IL-31, play a significant role in adjusting inflammatory response in many diseases; analysis of cytokines on genetic or serum expression level has been utilized to determine the susceptibility and outcome of diseases. In the present study, we explored the potential role of *IL-31* gene in the development of cryptorchidism and successfully identified a significant association between *IL-31* gene SNPs and susceptibility to cryptorchidism. The significant differences in the frequencies of allele and genotype in *IL-31* gene SNPs between patients with cryptorchidism and controls were also observed. Our findings suggest that the C allele of the rs4758680 locus could be the main predisposing risk factor. Moreover, A carriers (C/A-A/A genotype) of the rs4758680 locus may serve as a novel protection factor for cryptorchidism in the Chinese population.

In summary, our experimental evidence supports the assumption that IL-31 may play an indispensable role in the development of cryptorchidism. By providing novel knowledge about the pathogenesis of cryptorchidism and new insights into the potential role of IL-31 in cryptorchidism, the present study could potentially contribute to further investigations on the role of IL-31 in cryptorchidism and on its pathophysiology.

5. Limitations

Our empirical findings suggest that rs4758680 polymorphisms of *IL-31* may become a useful prediagnostic indicator for patients with cryptorchidism. Since the limited study subjects may lead to the potential of certain statistically significant results by chance, further study with a large scale and different ethnic populations of sample size as well as animal experiments should be performed to consolidate the conclusion. Moreover, research centering on the expression level of *IL-31* must be conducted to confirm whether SNPs in *IL-31* could change the serum level of *IL-31* and the possible association between serum *IL-31* level and cryptorchidism risk. Finally, scholars must elucidate the molecular mechanisms through which SNPs of *IL-31* are involved in altering susceptibility to cryptorchidism and finally establish approaches for the management of this disease.

Author contributions

BZ performed the PCR-RFLP experiment and drafted the manuscript. ZY, the co-contributing author, also performed the PCR-RFLP experiment. JH, the co-corresponding author, provided guidance in the study and helped with sample collection and in performing PCR-RFLP. CT carried out statistical data analysis, drafted the Word version, and created the tables. HW collected samples and extracted DNA samples from patients with cryptorchidism and healthy controls. JF partly helped with sample collection and primer design. XL helped in PCR-RFLP experiments and set up of the preliminary experiment to improve the experiment conditions. XW helped with experimental data collection. SC helped with data analysis and assisted in drafting the manuscript. TT, the corresponding author, conceived the study, and guided the whole procedure. All authors read and approved the final manuscript.

Data curation: Jing Huang, Xiaojun Wang.
Formal analysis: Chunlin Tan.
Investigation: Bing Zou, Zhihai Yu, Haiyun Wang, Xin Li.
Methodology: Jing Huang, Jian Fu.
Supervision: Tielong Tang.
Validation: Shu Cui.
Writing – original draft: Bing Zou.
Writing – review & editing: Shu Cui, Tielong Tang.

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