Ukrainian Journal of Nephrology and Dialysis
Scientific and Practical, Medical Journal

Founder:
- National Kidney Foundation of Ukraine

ISSN 2304-0238;
eISSN 2616-7352

Journal homepage: https://ukrjnd.com.ua

Research paper

doi: 10.31450/ukrjnd.3(83).2024.06

Anwar Abed Nasser Dhabaan[1], Hussein Mahmood Abbas[2], Haidar J. Muhammed[3], Tahreer Hadi Saleh[3]

Identification of the IL-13 gene rs20541 single nucleotide polymorphism and its association with renal cell carcinoma in Iraqi patients

[1]Department of Biology, College of Education, Al-Iraqia University, Baghdad, Iraq
[2]Department of Applied Pathological Analysis, College of Science, Al-Nahrain University, Baghdad, Iraq
[3]Department of Biology, College of Science, University of Mustansiriyah, Baghdad, Iraq

Citation: Dhabaan AAN, Abbas HM, Muhammed HJ, Saleh TH. Identification of the IL-13 Gene rs20541 single nucleotide polymorphism and its association with renal cell carcinoma in Iraqi patients. Ukr J Nephrol Dial. 2024;3(83):41-50. doi: 10.31450/ukrjnd.3(83).2024.06.

Abstract. *Renal cell carcinoma (RCC) is a common and aggressive form of kidney cancer, with genetic factors playing a significant role in its development. Recent research has focused on the potential involvement of cytokine gene polymorphisms, including those in the interleukin (IL) -13 gene, in RCC susceptibility. The present study aimed to investigate the association between the IL-13 gene rs20541 single nucleotide polymorphism (SNP) and the risk of RCC in Iraqi patients.*
Methods. A case-control study was conducted involving 250 participants, including 125 patients diagnosed with RCC and 125 healthy controls matched for age and sex. Blood samples were collected from all participants for DNA extraction. The IL-13 gene rs20541 SNP was genotyped using the Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain Reaction (TARMS-PCR) method. Statistical analysis was performed to compare the frequencies of genotypes (GG, GA, AA) and alleles (G, A) between cases and controls, and to evaluate their association with RCC risk.
Results. The study found significant differences in the distribution of IL-13 rs20541 genotypes and alleles between RCC patients and healthy controls. The presence of the GG genotype was significantly associated with an increased risk of RCC (OR = 3.7, 95% CI = 2.15–6.37, P = 0.002), while the AA genotype was significantly associated with a protective effect against the disease (OR = 0.38, 95% CI = 0.21–0.69, p = 0.002). On the other hand, the GA genotype did not show a significant association with protection against RCC (OR = 0.6, 95% CI = 0.36–1.00, p = 0.067). For the G and A alleles, the G allele was found to be a highly significant risk factor for RCC (OR = 2.67, 95% CI = 1.85–3.84, p <0.0001), while the A allele was significantly associated with a protective effect (OR = 0.37, 95% CI = 0.26–0.54, p <0.0001).
Conclusion. The current study provides evidence that the IL-13 rs20541 SNP, particularly the GG genotype and G allele, may be associated with an increased risk of developing RCC in the Iraqi population. This finding suggests that the rs20541 polymorphism could serve as a potential genetic marker for RCC susceptibility. Further research is needed to confirm these results and to explore the underlying mechanisms of how IL-13 gene polymorphisms contribute to RCC pathogenesis.
Keywords: *rs20541 single nucleotide polymorphism, renal cell carcinoma, interleukin-13 gene.*

Article history:
Received June 17, 2024
Received in revised form August 13, 2024
Accepted August 18, 2024

Conflict of interest. The authors declare no conflict of interest.

Correspondence should be addressed to Tahreer Hadi Saleh:
dr.tahreer80@uomustansiriyah.edu.iq

УДК: 616.61-006.6:575.113](567)

Анвар Абед Насер Дхабан[1], Хусейн Махмуд Аббас[2], Хайдар Дж. Мухаммед[3], Тахрір Хаді Салех[3]

Ідентифікація поліморфізму rs20541 гена IL-13 та його асоціація з нирково-клітинною карциномою у пацієнтів з Іраку

[1]Факультет біології, Педагогічний коледж, Університет Аль-Іракія, Багдад, Ірак
[2]Відділ прикладного патологічного аналізу, Науковий коледж, Університет Аль-Нахрейн, Багдад, Ірак
[3]Кафедра біології, Науковий коледж, Університет Мустансірія, Багдад, Ірак

Резюме. *Нирково-клітинний рак (НКР) є поширеною та агресивною формою раку нирки, у розвитку якої значну роль відіграють генетичні фактори. Останні дослідження зосереджені на потенційній участі поліморфізмів генів цитокінів, у тому числі в гені інтерлейкіну (ІЛ)-13, у сприйнятливості до НКР. Це дослідження мало на меті дослідити зв'язок між поліморфізмом rs20541 гена IL-13 і ризиком НКР у пацієнтів з Іраку.*

Методи. Нами було проведено дослідження «випадок-контроль» за участю 250 учасників, у тому числі 125 пацієнтів з діагнозом НКР та 125 здорових осіб контрольної групи відповідного віку та статі. В усіх учасників були відібрані зразки крові для виділення ДНК. Поліморфізм гена ІЛ-13 rs20541 генотипували за допомогою полімеразної ланцюгової реакції із системою ампліфікації рефрактерних мутацій із чотирма праймерами (Tetra-Primer ARMS-PCR). Статистичний аналіз проведили для порівняння частоти генотипів (GG, GA, AA) і алелів (G, A) між випадками та контролем, а також для оцінки їх зв'язку з ризиком НКР.

Результати. Визначено статистично значущі відмінності в розподілі генотипів і алелів IL-13 rs20541 між пацієнтами з НКР і здоровими. Наявність генотипу GG була достовірно асоційована з підвищеним ризиком НКР (OR = 3,7, 95% ДІ = 2,15–6,37, P = 0,002), тоді як генотип AA був асоційований з захисним ефектом проти НКР (OR = 0,38, 95% ДІ = 0,21–0,69, p = 0,002). З іншого боку, генотип GA не показав значущого зв'язку (OR = 0,6, 95% ДІ = 0,36–1,00, p = 0,067). Для алелей G і A було виявлено, що алель G є значущим фактором ризику НКР (OR = 2,67, 95% ДІ = 1,85–3,84, p <0,0001), тоді як алель A був значно асоційованим з захисним ефектом (OR = 0,37, 95% ДІ = 0,26–0,54, p <0,0001).

Висновки. Представлене дослідження свідчить, що поліморфізм rs20541 гена IL-13, зокрема генотип GG і алель G, можуть бути пов'язані з підвищеним ризиком розвитку НКР в популяції Іраку. Поліморфізм rs20541 може служити потенційним генетичним маркером сприйнятливості до НКР. Подальші дослідження необхідні для підтвердження отриманих результатів та вивчення основних механізмів впливу поліморфізму гена IL-13 на патогенез НКР.

Ключові слова: *поліморфізм rs20541, нирково-клітинний рак, ген інтерлейкіну-13.*

Introduction. Eighty to eighty-five percent of all primary renal neoplasms are renal cell carcinomas (RCCs), also known as Grawitz tumors or hypernephroma, which are derived from the renal cortex [1]. In Iraq, the incidence rate of RCC is 1-4% [2]. Globally, RCCs are the 12th, 9th, and 7th most common cancer in Europe, worldwide, and the United States respectively [3] and [4]. Innate and adaptive immune cells, as well as non-immune cells and tissues, communicate through interleukins and related cytokines. Interleukins have a crucial role in the initiation, advancement, and management of cancer. By utilizing these characteristics of interleukins, immunotherapies can be enhanced to increase efficacy and reduce side effects [5]. Additionally, IL-13 has been demonstrated to be involved in the immunopathogenesis of the disease. IL-13 is located on human chromosome 5q31 and shares 25% of its DNA with IL-4 [6].

Numerous investigations revealed that IL-13 is produced by a variety of cells, including fibroblasts, natural killer cells, and eosinophils, and has a wide range of biological activities [7]. This protein is considered a multi-functional peptide, such as its role as an anti-inflammatory and at the same time a negative regulation of tumor control and an inhibitor of immunotherapies and cancer treatments [8] and [9] as well as IL-13 and its receptors can also stimulate tumor-associated macrophages, causing metastasis [10-12]. The genetic variants of IL-13 have been associated with the risk of RCC. The rs20541 polymorphism was found to affect the RCC, increase the secreting ability of other cytokines, and exacerbate the risk of RCC [13]. In addition, [14] demonstrated that the rs20541 polymorphism genotypes increased the risk of lymph node metastasis. This SNP causes the positively charged arginine to be replaced with the neutral glutamine, which causes the activation of IL-13 transcription [15]. The meta-analysis of [16] findings suggested that rs20541 polymorphism may contribute to susceptibility to cancer, especially for glioma. On the other

Tahreer Hadi Saleh:
dr.tahreer80@uomustansiriyah.edu.iq

hand, [17] clarified that the rs20541 polymorphism may lower the risk of RCC occurrence. Human health is influenced by a variety of factors, including genetic inheritance, behavioral lifestyle, socioeconomic and environmental situations, and the public availability of treatment and therapies in the event of disease, with the assistance of the national health system. All of these variables are the foundation for preventing and promoting a healthy lifestyle [18]. Evidently, the disparities between studies with regard to the outcomes are caused by these crucial criteria in diagnosing and evaluating the condition in a specific population group in an area.

The current study aimed to find out whether an association exists between the rs20541 polymorphism in the IL-13 gene and RCC in the Iraqi population, especially the shortage of studies on the subject.

Material and methods. ***Study design and participants.*** A case-control study was undertaken to investigate the potential association between the rs20541 genotypes of IL-13 and the development of RCC in Iraqi patients. There were 250 participants in this study; half were patients, and the other half served as a comparison control group. In the current work, 125 individuals participated, with a mean age of 57 years old. The cases were diagnosed by specialists at Baghdad Medical City Hospital for Cancer Diseases. Based on clinical examination by the specialist physician and laboratory tests, RCC was confirmed for all cases.

Control selection. In this study, the control group was carefully selected to avoid bias or confounding. With the same number of patients participating, 125 participants were collected in this study as a control group. Some important criteria were selected for determining the control group sample. These criteria were represented by the average age of the participants, which was approximately 52 years, which is a relatively old age for disease occurrence. They did not have a family history of the disease and did not suffer from other health problems or comorbidities, and their health was generally good.

Specimen collection. About 3ml of venous blood was collected from patients as well as controls. Around three ml of aspirated blood was collected from each participant and immediately transferred into an EDTA tube and mixed well for DNA extraction. The samples were kept frozen (-20°C) during the period of sample collection until they were used for DNA extraction.

Detection of rs20541 polymorphism in the IL-13 gene. According to the recommendation of the manufacturer, a DNA genomic kit (Bioneer/Korean) was used for DNA extraction. A nanodrop was used to measure the DNA purity, which was found to be between 1.6 and 1.8. Until usage, all samples were maintained at minus 20 degrees.

The primer selection and preparation. The specific primers (Alpha DNA, Canada) in lyophilized form were used in the current work to detect the rs20541 polymorphism genotypes and IL-13 gene. These primers were diluted in sterile, nuclease-free water to get a final concentration of 100μM, then kept at -20°C until use. A 10M concentration was created and used quickly if necessary. The selection primers were designed via [6] for the detection of the rs20541 genotypes. An ARMS-PCR reaction was performed on a DNA template for each allele and genotype. The primers shown in Table 1 were used to amplify the DNA sequences under investigation.

Table 1

Primer sequences of the IL-13 gene as well as rs20541 polymorphism by using TARMS-PCR

Target Gene	Primer	Primer sequences (5`→ 3`)	Size (bp)
IL-13 gene (rs20541 G>A)	A allele: Inner F	GAAACTTTTTCGCGAGGGCCA	108
	G allele: Inner R	GATGCTTTCGAAGTTTCAGTTGACCC	168
	Outer F	CTAACAGTACCCACCTCATGGGGACTT	280
	Outer R	GAAGGCTGAGGTCGGCTAGGCT	

Tetra-primer ARMS PCR. The outer forward and reverse primers were used to identify the IL-13 gene. A total of 20μl volume of the PCR reaction was prepared, which included 10μl of master mix (Quick-Load® Taq 2X), 2μl of each outer forward and reverse primers (10μM), 5μl of DNA template, and nuclease-free water, which were added to the PCR mixture to get a final volume of 20μl. The product size of two outer primers was 280 bp. When detecting the IL-13 gene, the final product of the PCR reaction was used in order to detect rs20541 polymorphisms by using two inner-specific primers (Table 1). After being briefly stirred by vortex, the Eppendorf tubes were put into PCR. The final volume of the PCR was 20μl. TARMS-PCR cycling conditions are summarized as follows: initial denaturation at 94°C for 5min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 2min, and extension at 72°C for 40 s, followed by a final extension step at 72°C for 5min. An electrophoresed 2% agarose gel stained with ethidium was used to determine the genotypes. The lengths of the G and A alleles were 168 bp and 108 bp, respectively.

Statistical analysis. Data were analyzed using IBM SPSS version 25.0 to assess the normality of the distribution, with results presented as mean ± SE. Fisher's exact test, with a significance level set at $P<0.05$, was used to evaluate the association between rs20541 genotypes and renal cell carcinoma compared

to control groups. This test is particularly suitable for categorical data organized in contingency tables, such as case-control studies, to determine whether there is a significant difference between proportions or a correlation between variables. Odds ratios (OR) and confidence intervals (CI) were calculated using the WinPepi program. The results were also compared against the Hardy-Weinberg equilibrium to ensure consistency.

Ethical Approval. The study was conducted in accordance with the Helsinki Declaration. All participants provided informed consent to donate blood samples and share relevant information. The study was also approved by the Ethics Committee of the Oncology Teaching Hospital, Baghdad Medical City, Iraq, under reference number 5826/5-11-2023.

Results. The study participants' characteristics. In the present work, 250 people participated, distributed into two groups, half of which represented the case group and the other half representing the control group.

Table 2 illustrates the main relevant factors considered in this study, such as age, sex, and comorbidities.

Table2

Demographic characteristics of the studied groups

Characteristics	Mean ± SE		p-value
	Control	Cases	
Age	52.9±1.04	57.62±1.14	0.002
Sex (male)	51.24±1.4	56.91±1.4	0.005
Sex (female)	55±1.5	59.65±1.7	0.06

There were significant differences in age distribution between the patient group and the control group (P = 0.002). Additionally, significant differences were observed between patients and controls among men (P = 0.005), while no significant differences were found among women (P = 0.06). The ages of the study participants ranged from 25 to 80 years. Figure 1 illustrates the percentage distribution of patients by age group, including both cases and controls. The results indicated that the highest proportion of renal cell carcinoma cases (28%) occurred in the 50-year age group. The incidence rates for other age groups were as follows: 23.2% in the 60s, 20.8% in the 40s, 16% in the 70s, 4.8% in the 30s, 4% in the 80s, and 3.2% in the 20s.

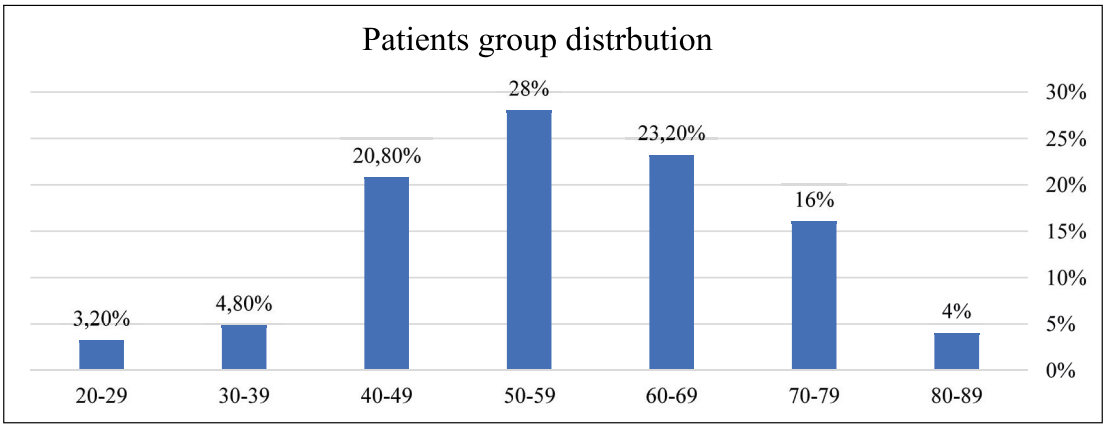

Fig 1: The percentage of patients group according to age.

Among the 93 male participants, 50 (53.76%) had a family history of the illness, while 19 out of 32 female participants (59.37%) reported a family history of the disease (Table 3).

Table 3

Family history and comorbidities among male and female participants

Characteristics	Total participant (125)			Percentage
	Total		History of disease	
History of disease	Male	93	50	53.76%
	Female	32	19	59.37%
Comorbidities	Male	No related		-
	Female	No related		-

No comorbidities were observed in any participants, as confirmed by laboratory findings and interviews regarding their health status. Information on tumor stage, metastasis, and treatment methods was not available, as these details fall under the purview of the treating specialist and could not be obtained.

Detection of rs20541 polymorphism. In Fig. 2, the specific band locations for the A and G alleles of the rs20541 polymorphism, as well as the IL-13 gene, are shown at 108 bp, 168 bp, and 280 bp, respectively. The results depict both the G and A alleles in the rs20541 SNP and the IL-13 gene in the case and control groups.

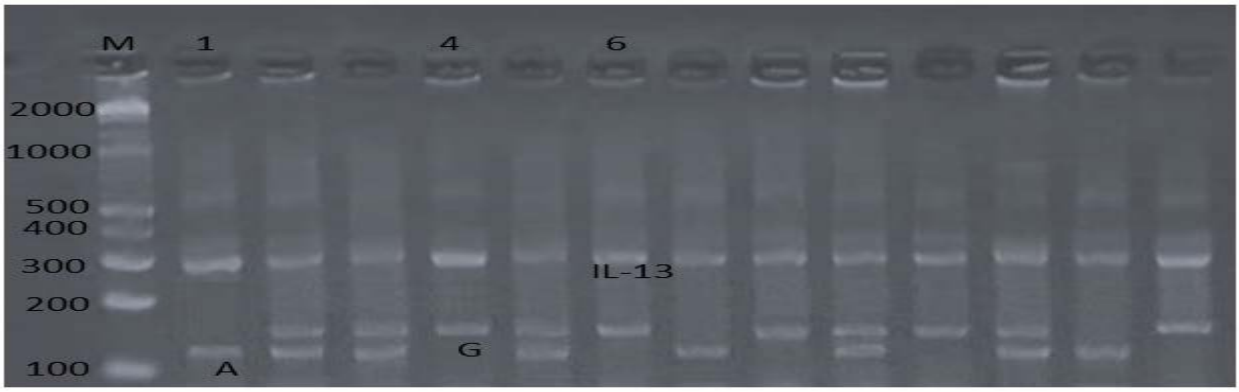

Fig. 2. Tetra-Primer ARMS-PCR amplification of rs20541 polymorphism in the IL-13 gene. The figure illustrates the results of Tetra-Primer ARMS-PCR amplification of the polymorphic alleles of the rs20541 polymorphism in the IL-13 gene. The PCR products were separated on a 1.5% agarose gel stained with 0.5 mg/ml ethidium bromide and electrophoresed at 120 V for 80 minutes. Bands were visualized under UV light. The M lane contains a 100-bp molecular-weight DNA marker. Lanes 1, 4, and 6 correspond to the A allele, G allele, and IL-13 gene, respectively.

The Hardy-Weinberg equilibrium (HWE) was maintained, and the frequencies of heterozygote and homozygote alleles for each genotype were consistent. Genotype distribution and allele frequencies were analyzed using chi-square tests, with associations reported as odds ratios (OR) along with relative risk estimates and 95% confidence intervals (CI).

Table 4 details the genotype and allele frequencies for the rs20541 polymorphism in the IL-13 gene in both patient and control groups. In the case group, the GG genotype was the most common (52.8%), compared to the AG (31.2%) and AA (16%) genotypes. Conversely, the control group showed a higher percentage of the AG genotype (43.2%), with the AA and GG genotypes at 33.6% and 23.2%, respectively.

Table 4

The genotype frequencies of rs20541 polymorphisms in the IL-13 gene in healthy and RCC samples

Genotype	Cases (n=125)				Control (n=125)				OR (95%CI)	RF	P
	Observed		Excepted		Observed		Excepted				
	No	%	no	%	no	%	no	%			
AA	20	16%	12.48	10%	42	33.6%	38.09	30.47%	0.38 (0.21-0.69)	0.21	0.002
AG	39	31.2%	54.04	43.2%	54	43.2%	61.82	49.46%	0.6 (0.36 - 1.00)	0.17	0.067
GG	66	52.8%	58.48	46.8%	29	23.2%	25.09	20.07%	3.7 (2.15 - 6.37)	0.38	<0.0001
Total	125	100	125	100	125	100	125	100			
P-HWE	0.001				0.15						

Notes: OR= Odds ratio, CI= Confidence Interval, RF=Risk factor, P<0.05 by Fisher's test

The OR for the GG genotype was 3.7 (95% CI = 2.15–6.37), indicating a significant risk factor (0.38) with a highly significant p-value (P = 0.0000002). The OR for the AG genotype was 0.6 (95% CI = 0.36–1.00), suggesting a less significant protective factor (0.17) with a p-value of 0.067. For the AA genotype, the OR was 0.38 (95% CI = 0.21–0.69), indicating a significant protective factor (0.38) with a p-value of 0.002.

Besides, the OR of G and A alleles were 2.67 (95% CI = 1.85 - 3.84) and 0.37 (95% CI = 0.26 - 0.54), respectively, in Table 5. The G allele risk factor (0.42) is highly significant (P = 0.0000001) and would be considered an etiological factor, whereas the A allele risk factor (0.2) is a highly significant (P = 0.0000001) protective factor. Based on the above results, the GG genotypes and G alleles are associated with renal cell carcinoma.

Table 5

Allele frequencies of polymorphisms in IL-13 gene (rs20541 G>A) genes in controls and RCC patients

Target Gene	Allele	Renal cell carcinoma Number (%)	Control Number (%)	OR (95%CI)	RF	P
IL-13 gene (rs20541 G>A)	G	171 (68.4%)	112(44.8%)	2.67 (1.85 - 3.84)	0.42	<0.0001
	A	79(31.60%)	138(55.2%)	0.37 (0.26 - 0.54)	0.35	<0.0001

*Notes: OR= Odds ratio, CI= Confidence Interval, RF=Risk factor, P<0.05 by Fisher's test, ** highly significant*

Table 6 illustrates the distribution of age groups according to their genotypes in cases compared to control. Table 4 explains the significant differences between patient and control groups in age. Furthermore, this table presents significant differences (P = 0.0001) between patients and controls in AA genotypes in the male group only. Non-significant differences were noted between other genotypes in the male and female groups. This result may not reflect a clear association between the disease incidence in elderly age groups and their genotypes.

Table 6

Distribution of age groups according to their genotypes

Sex	Genotypes	(Mean ± SE) control	(Mean ± SE) cases	P-value
Male	AA	(47.33±1.92)	(58.1±1.88)	0.0001
	AG	(54.54±2.48)	(55.48±2.37)	0.7
	GG	(53.86±2.96)	(56.88±2.62)	0.4
Female	AA	(52.1±1.72)	(60.11±2.39)	0.2
	AG	(54.3±2.78)	(60.8 ± 3.86)	0.1
	GG	(58.1±2.15)	(61.751±1.98)	0.1

The results in Table 7 illustrate the distribution of patient groups according to sex. Moreover, non-significant differences P = 0.19, 0.25, and 0.8 were noticed in the AA, AG, and GG genotypes, respectively. Although the GG genotype and the G allele have been linked as a risk factor for the disease incidence, it cannot be said that there is a direct link to the development of the disease according to the genotype for either sex.

Table 7

The genotype distribution according to sex

Genotypes	(Mean ± SE)		P-value
	Male	Female	
AA	(58.1±1.88)	(61.751±1.98)	0.19
AG	(55.48±2.37)	(60.8 ± 3.86)	0.25
GG	(56.88±2.62)	(57.64 ±2.9)	0.8

Discussion. El-Derany found that the rs20541 polymorphism is a significant contributor to cancer progression in non-alcoholic steatohepatitis [19]. According to [20], there may be a weak link between glioma susceptibility and the IL-13 gene rs20541 polymorphism, and the associations may vary by ethnicity. Moreover, the rs20541 polymorphism of IL-13 was related to the risk of non-small lung cancers among African-Americans but not among Caucasians [21]. The IL-13 polymorphisms may be linked with renal cell carcinoma, as indicated by the results of the current study, and this was previously confirmed by the study of [22], who reported that a reduction in IL-13 production may result in tumors that are less likely to recur. Other studies were also recorded that the polymorphisms of IL-13 associated with various human diseases, including; colorectal cancer, bladder cancer, breast cancer, and renal cell carcinoma [23, 24]. On the other hand, the results submitted by [25] noted that only the rs20541 SNP in between ten SNPs was significantly (P = 0.0054) associated with the decreased risk of renal cell carcinoma in Chinese populations. Moreover, the findings of the current work illustrated incompatibility with those recorded by [26], who have suggested that the carriers of the rs20541 polymorphism were significantly associated with reduced cancer risk. [27] recorded that

the rs20541 polymorphism of IL-13 was not associated with gastrointestinal cancer risk for any of the genetic models and subgroup analyses.

Many cells are expressed for IL-13, such as T-helper 2 cells, so IL-13 was secreted from activated T-helper 2 cells and works as a regulatory cytokine of humoral immunity as well as cytokines that mediate immune response type II with many biological functions, including inflammatory immune response promotion and inhibition of inflammatory cytokine production [28]. Using a genome-wide association investigation, Urayama's findings revealed that there is one SNP at chromosomal region 5q31 (IL13, rs20541) and other 18SNPs in the MHC region, chosen from among >100 genome-wide significant MHC region SNPs [40]. This may be one of the most important reasons for the biological association of this SNP with the disease. The mechanism of the occurrence of disorders associated with IL-13 may be related to the numerous impacts in regard to the regulation and induction of inflammation caused by the immunological and biological role of IL-13 in immune and inflammatory regulation [29].

As mentioned previously, the location of the MHC rs20541 SNP and its functional significance in the region may be a potential risk factor for the pathogenesis of some diseases. On the other hand, Th1, Th2, and Th17 play an important role in the immune system, which is regulated by IL-13, so these may indicate the importance of IL-13 in the immune system [30]. Furthermore, [31-33] detected the evolution of IL-13 levels in breast cancer, colorectal cancer, and oral squamous cell carcinoma respectively. Due to its pleiotropic effects on inflammation and immunoregulation, IL13 may have a role in the development of cancer. It was recently reported that in mouse cancer models, IL13 induced myeloid cells to produce transforming growth factor-β, which mediated cancer immunosurveillance. In bladder cancer patients, however, IL13 production was observed to be elevated as an apparent immune response against inflammation [34, 35]. Consequently, it is reasonable to assume that IL13 variations will impact carcinogenesis through their impact on cancer immune responses. The variation between the above studies may come back to sample size, geographic area, lifestyle of population understudies, and genetic predisposition for some groups. Furthermore, the frequency of RCC rises gradually with age [36, 29]. These findings supported those of the present investigation, demonstrating that the elderly had the highest infection rates. The recurrence or incidence of the disease in individuals after treatment may be a third since the disease recurs in a metastatic form [37].

In the current results, the findings explain that the rate of disease incidence in males reaches 74.4% than in females 25.6%. Similar findings were reported by [38], who asserted that men experience disease incidence at a rate twice as high as that of women. There is evidence to imply that individual characteristics, including hormones and heredity, are the cause of this discrepancy in male incidence. In addition, men suffer from larger and more aggressive tumors than women. Female hormones like estrogen may have a preventive effect on kidney cancer, which is one theory that helps to explain the situation partially Trusted Source. Women tend to be older than men when kidney cancer is diagnosed, which may be explained by the declining amounts of estrogen in elderly women [38]. According to an analysis of the United States Cancer Statistics (USCS) public use database from 2001 to 2016, for a wide range of cancer sites, including the bladder, kidney, colorectum, liver, esophagus, head and neck, brain, skin, and blood, men have a greater incidence and worse survival outcomes than women [39]. Moreover, it has not been observed that the development of the disease is genetically linked to sex, this may be due to the fact that the disease is multifactorial such as genetic, immunological, and environmental factors [25].

Genetic testing is used for screening, diagnosis, and prognosis of disorders compatible with a genetic etiology, as well as to guide pharmacological therapy to optimize drug efficacy and reduce side effects. In practice, this attempts to inform about the availability of DNA-related genetic tests, their interpretation, and recommended clinical interventions based on the results, using evidence from clinical recommendations when available. Some of the challenges associated with the adoption of genetic testing for patient diagnosis and treatment include the lack of demonstrated clinical validity and utility of some genetic variants (such as the majority of the genetic variants identified in genome-wide association studies) and the availability of less expensive alternatives (e.g., dosing blood levels of the drug rather than adjusting based on genetic testing).

The current study has to overcome a few challenges. Firstly, only one hospital was used to enroll the RCC patients and controls, therefore their representation of the whole population may be limited. Furthermore, while the polymorphisms examined in this study were selected based on their functional significance, future research should look for markers in other genes that may be able to predict an individual's susceptibility to RCC. Third, this study's statistical power was constrained by its moderate sample size. Lastly, additional research is required to validate our results and elucidate the genetic process underlying RCC, particularly in relation to the gene-environment interaction. Practical applications for such outcomes include early genetic discovery of genes, risk factor identification through investigations, and follow-up immunological testing. There must also be education about the most essential elements contributing to the disease's development, such as smoking and continuous exposure to carcinogenic stimuli, as well as excessive alcohol intake and other risk factors. Finally, the PCR was applied in human illnesses and genetic diseases [41-51], for that we can suggest using these molecular techniques in different medical areas.

Conclusions. This study identified a significant association between the rs20541 polymorphism in the

IL-13 gene and RCC in an Iraqi population. Specifically, the GG genotype and G allele were found to be strong risk factors for RCC, with a notable odds ratio of 3.7 (95% CI: 2.15–6.37) for the GG genotype and 2.67 (95% CI: 1.85–3.84) for the G allele. Conversely, the AA genotype and A allele were associated with a protective effect against RCC, suggesting a potential role of IL-13 genetic variants in modifying cancer risk. Further research with larger sample sizes and diverse populations is needed to validate these results and explore the underlying mechanisms by which IL-13 influences RCC development.

Funding source. The research was entirely self-funded by the authors.

Data availability. The data used in this study are available from the corresponding author and all co-authors upon request.

Competing interests. The authors declare that they have no competing interests.

The author's contributions. All the authors contributed to proposing the research plan, writing the manuscript, analyzing the results statistically, and implementing the research plan.

References:

1. *Kuroda N, Sugawara E, Kusano H, Yuba Y, Yorita K, Takeuchi K.* A review of ALK-rearranged renal cell carcinomas with a focus on clinical and pathobiological aspects. Polish journal of pathology: official journal of the Polish Society of Pathologists.2018; 69(2):109–113. doi:10.5114/pjp.2018.76693.
2. *Zamzami Z.* The Risk Factors of Renal Cell Carcinoma Patients in Arifin Achmad Regional General Hospital, Riau Province, Indonesia. South-East Asia Journal of Medical Sciences. 2018;2(2):1-4. doi:10.5281/zenodo.2547628.
3. *Tahbaz R, Schmid M, Merseburger AS.* Prevention of kidney cancer incidence and recurrence: lifestyle, medication and nutrition. Curr Opin Urol. 2018;28(1): 62-79. doi: 10.1097/MOU.0000000000000454.
4. *Sidon MA, Albolatti KA, Elfageih MA, Elturki AA, Elrgaig MA, salah KSb, et al.* Epidemiological Characters of Renal Malignancies in Midland Region of Libya; Single Center Experience (National cancer institute Misrata Libya). Lebda Medical Journal.[Internet].2021;7(1):244-251. Available from: https://lebmedj.elmergib.edu.ly/index.php/LMJ/article/view/137.
5. *Briukhovetska D, D rr J, Endres S, Libby P, Dinarello CA, Kobold S.* Interleukins in cancer: from biology to therapy. Nat Rev Cancer. 2021;21(8):481-499. doi: 10.1038/s41568-021-00363-z.
6. *Modher Nabat Al-Ajrash A, Mohammed Ali SH, Al-Bayaa YJ, MohammedAl-Alwany SH, Al-Hijazi AY.* Assessment of Interleukin-13(rs20541) Genomic Polymorphism in Patients with Acute Respiratory Distress Syndrome in Relation to COVID19 Infection. Arch Razi Inst. 2022;77(6):2291-2298. doi: 10.22092/ARI.2022.358616.2266.
7. *Peebles RS Jr, Aronica MA.* Proinflammatory Pathways in the Pathogenesis of Asthma. Clinics in chest medicine. 2019;40(1):29–50. doi: 10.1016/j.ccm.2018.10.014
8. *Terabe M, Matsui S, Noben-Trauth N, Chen H, Watson C, Donaldson DD, et al.* NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. Nat Immunol. 2000;1(6):515-20. doi: 10.1038/82771.
9. *Gandhi NA, Pirozzi G, Graham NMH.* Commonality of the IL-4/IL-13 pathway in atopic diseases. Expert Rev Clin Immunol. 2017;13(5):425-437. doi: 10.1080/1744666X.2017.1298443.
10. *Ruffell B, Affara NI, Coussens LM.* Differential macrophage programming in the tumor microenvironment. Trends Immunol. 2012;33(3):119-26. doi: 10.1016/j.it.2011.12.001.
11. *Lin C, Liu H, Zhang H, He H, Li H, Shen Z, et al.* Interleukin-13 receptor α2 is associated with poor prognosis in patients with gastric cancer after gastrectomy. Oncotarget. 2016;7(31):49281–49288. doi: 10.18632/oncotarget.10297.
12. *Shi J, Song X, Traub B, Luxenhofer M, Kornmann M.* Involvement of IL-4, IL-13 and Their Receptors in Pancreatic Cancer. International journal of molecular sciences.2021;22(6):2998. doi: 10.3390/ijms22062998.
13. *Noguchi E, Nukaga-Nishio Y, Jian Z, Yokouchi Y, Kamioka M, Yamakawa-Kobayashi K, et al.* Haplotypes of the 5' region of the IL-4 gene and SNPs in the intergene sequence between the IL-4 and IL-13 genes are associated with atopic asthma. Human immunology. 2001;62(11):1251–1257. doi: 10.1016/s0198-8859(01)00338-x.
14. *Wang R, Qin HM, Liao BY, Yang FL, Wang JL.* Genetic polymorphisms in interleukin 13 gene with the susceptibility to nasopharyngeal carcinoma in a Chinese population. Cytokine. 2019; 115: 121–126. doi: 10.1016/j.cyto.2018.11.020.
15. *Vladich FD, Brazille SM, Stern D, Peck ML, Ghittoni R, Vercelli D.* IL-13 R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation. J Clin Invest. 2005;115(3):747-754. doi: 10.1172/JCI22818.

16. *Su T, Mi Y, Zhang L, Wang S, Lu H, Shi L, et al.* Association between IL13 gene polymorphisms and susceptibility to cancer: a meta-analysis. Gene. 2013;515(1):56-61. doi: 10.1016/j.gene.2012.11.035.
17. *Chu H, Wang M, Yan F, Zhong D, Shi D, Ma L, et al.* Polymorphisms in the IL-13 and IL-4R genes are associated with the development of renal cell carcinoma. Ann Oncol. 2012;23(8):2114-2121. doi: 10.1093/annonc/mdr607.
18. *Chiarella P, Capone P, Sisto R.* Contribution of Genetic Polymorphisms in Human Health. Int J Environ Res Public Health. 2023;20(2):912. doi: 10.3390/ijerph20020912.
19. *El-Derany MO.* Polymorphisms in Interleukin 13 Signaling and Interacting Genes Predict Advanced Fibrosis and Hepatocellular Carcinoma Development in Non-Alcoholic Steatohepatitis. B iology.2020;9(4):75. doi: 10.3390/biology9040075.
20. ***Yang D, Yuan Y, Zhang S, Zhao K, Li F, Ren H, et al.*** Association between IL-13 Gene rs20541 Polymorphism and Glioma Susceptibility: A Meta-Analysis. Oncol Res Treat. 2018;41(1-2):14-21. doi: 10.1159/000480518.
21. *Bao WL, Shi H, Zhang AQ, Kong XM, Deng DH, Zhang YJ.* Lack of associations of polymorphisms of IL-7R, IL-13 and IL-15 with NSCLCs in non-smoking Chinese. Asian Pac J Cancer Prev. [Internet].2011;12(12):3239-44. Available from: https://journal.waocp.org/article_26045_03ef0066013741480944866 90c00b12c.pdf.
22. *Terabe M, Park JM, Berzofsky JA.* Role of IL-13 in regulation of anti-tumor immunity and tumor growth. Cancer immunology, immunotherapy: CII. 2004;53(2):79-85. doi: 10.1007/s00262-003-0445-0.
23. *Walczak A, Przyby owska K, Trzci ski R, Sygut A, Dziki L, Dziki A, Majsterek I.* Association of -1112 c/t promoter region polymorphism of the interleukin 13 gene with occurrence of colorectal cancer. Pol Przegl Chir. 2011;83(1):27-31. doi: 10.2478/v10035-011-0004-x.
24. *Chu H, Ma L, Wang M, Shi D, Qin C, Yuan L, et al.* The polymorphisms of IL-4, IL-4R and IL-13 genes and bladder cancer risk in a Chinese population: a case-control study. Mol Biol Rep. 2012;39(5):5349-57. doi: 10.1007/s11033-011-1334-9.
25. *Liang G, Liu Q, Sun J, Liu W, Shen W.* Original Article The rs20541 G>A polymorphism in the interleukin 13 gene is associated with a decreased risk of renal cell carcinoma in a Chinese population. Int J Clin Exp Pathol. [Internet].2017;10(1):708-716. Available from: https://e-century.us/files/ijcep/10/1/ijcep0041115.pdf.
26. *Su T, Mi Y, Zhang L, Wang S, Lu H, Shi L, et al.* Association between IL13 gene polymorphisms and susceptibility to cancer: a meta-analysis. Gene. 2013;515(1):56-61. doi: 10.1016/j.gene.2012.11.035.
27. *Cho YA, Kim J.* Association of IL4, IL13, and IL4R polymorphisms with gastrointestinal cancer risk: A meta-analysis. J Epidemiol. 2017;27(5):215-220. doi: 10.1016/j.je.2016.06.002.
28. *Roy S, Schmeier S, Kaczkowski B, Arner E, Alam T, Ozturk M, et al.* Transcriptional landscape of Mycobacterium tuberculosis infection in macrophages. Sci Rep. 2018;8(1):6758. doi: 10.1038/s41598-018-24509-6.
29. *Wood N, Whitters MJ, Jacobson BA, Witek J, Sypek JP, Kasaian M, et al.* Enhanced interleukin (IL)-13 responses in mice lacking IL-13 receptor alpha 2. J Exp Med. 2003;197(6):703-9. doi: 10.1084/jem.20020906.
30. *Mao YM, Zhao CN, Leng J, Leng RX, Ye DQ, Zheng SG, Pan HF.* Interleukin-13: A promising therapeutic target for autoimmune disease. Cytokine Growth Factor Rev. 2019;45:9-23. doi: 10.1016/j.cytogfr.2018.12.001.
31. *Srabovici N, Mujagic Z, Mujanovic-Mustedanagic J, Muminovic Z, Softic A, Begic L.* Interleukin 13 expression in the primary breast cancer tumour tissue. Biochem Med (Zagreb). 2011;21(2):131-8. doi: 10.11613/bm.2011.021.
32. *Formentini A, Braun P, Fricke H, Link KH, Henne-Bruns D, Kornmann M.* Expression of interleukin-4 and interleukin-13 and their receptors in colorectal cancer. Int J Colorectal Dis. 2012;27(10):1369-76. doi: 10.1007/s00384-012-1456-0.
33. *Aziz S, Ahmed SS, Ali A, Khan FA, Zulfiqar G, Iqbal J, et al.* Salivary Immunosuppressive Cytokines IL-10 and IL-13 Are Significantly Elevated in Oral Squamous Cell Carcinoma Patients. Cancer Invest.2015;33(7):318-28. doi: 10.3109/07357907.2015.1041642.
34. *MalekZadeh K, Nikbakht M, Sadeghi IA, Singh SK, Sobti RC.* Overexpression of IL-13 in patients with bladder cancer. Cancer Invest. 2010;28(2):201-7. doi: 10.3109/07357900903181977.
35. *Ziyab AH, Davies GA, Ewart S, Hopkin JM, Schauberger EM, Wills-Karp M, et al.* Interactive effect of STAT6 and IL13 gene polymorphisms on eczema status: results from a longitudinal and a cross-sectional study. BMC Med Genet. 2013;14:67. doi: 10.1186/1471-2350-14-67.
36. *Bai H, Jiang W, Wang D, Shou J, Li C, Xing N.* Efficacy and safety of surgery in renal carcinoma patients 75 years and older: a retrospective analysis. BMC Urol. 2022;22(1):135. doi: 10.1186/s12894-022-01088-3.
37. *Mathis S, Sierpina VS.* Kidney Cancer Integrative Oncology: Possible Options for Care. Curr Oncol Rep. 2023 Sep;25(9):1071-1080. doi: 10.1007/s11912-023-01437-x.

38. *Scelo G, Larose TL.* Epidemiology and Risk Factors for Kidney Cancer. J Clin Oncol. 2018;36(36):JCO2018791905. doi: 10.1200/JCO.2018.79.1905.

39. *Dong M, Cioffi G, Wang J, Waite KA, Ostrom QT, Kruchko C, et al.* Sex Differences in Cancer Incidence and Survival: A Pan-Cancer Analysis. Cancer Epidemiol Biomarkers Prev. 2020;29(7):1389-1397. doi: 10.1158/1055-9965.EPI-20-0036.

40. *Urayama KY, Jarrett RF, Hjalgrim H, Diepstra A, Kamatani Y, Chabrier A, et al.* Genome-wide association study of classical Hodgkin lymphoma and Epstein-Barr virus status-defined subgroups. J Natl Cancer Inst. 2012 Feb 8;104(3):240-53. doi: 10.1093/jnci/djr516.

41. *Bassi AG, Al-Rubaii BA.* Detection of Pyocin S and the Effects of Lactobacillus Acidophilus Cell-Free Supernatants on Multi-Drug Resistant Pseudomonas Aeruginosa Isolated from Patients of Baghdad Hospitals. Journal of Communicable Diseases. 2024; 56(1):135-144. doi: 10.24321/0019.5138.202418.

42. *Al-Jumaily RM, AL-Sheakli II, Muhammed HJ, Al-Rubaii BA.* Gene expression of Interleukin-10 and Foxp3 as critical biomarkers in rheumatoid arthritis patients. Biomedicine. 2023;43(4):1183-7. doi: 10.51248/.v43i4.3107.

43. *Sabah Fakhry S, Noori Hammed Z, Abdulelah Bakir W, Abdullah Laftaah ALRubaii B.* Identification of methicillin-resistant strains of Staphylococcus aureus isolated from humans and food sources by Use of mecA 1 and mecA 2 genes in Pulsedfield gel electrophoresis technique. Bionatura. 2022;7(2):44. doi: 10.21931/RB/2022.07.02.44.

44. *Ali SM, Laftah BA, Al-Shammary AM, Salih HS.* Study the role of bacterial neuraminidase against adenocarcinoma cells in vivo. AIP Conference Proceedings. 2021;2372(1): 030009. doi: 10.1063/5.0067193.

45. *Salih HS, Al-Shammari AM, Laftaah BA.* Intratumoral co-administration of oncolytic newcastle disease virus and bacterial hyaluronidase enhances virus potency in tumor models. Journal of Global Pharma Technology. [Internet]. 2018; 10(10):303-10. Available from: https://www.researchgate.net/publication/331075531_Intratumoral_Co-Administration_of_Oncolytic_Newcastle_Disease_Virus_and_Bacterial_Hyaluronidase_Enhances_Virus_Potency_in_Tumor_Models.

46. *Muhsin HY, Al-Humairi RM, Alshareef DQ, Ad'hiah AH.* Interleukin-22 is up-regulated in serum of male patients with ankylosing spondylitis. The Egyptian Rheumatologist. 2022;44(4):351-355. doi: 10.1016/j.ejr.2022.07.002.

47. *Bresam S, Alhumairi RM, Hade IM, Al-Rubaii BA.* Genetic mutation rs972283 of the KLF14 gene and the incidence of gastric cancer. Biomedicine. 2023; 43(4):1256-60. doi: 10.51248/.v43i4.3112.

48. *Saleh TH, Hashim ST, Abdulrazaq Al-Obaidi RA, AL-Rubaii BA.* A biological study of chitinase produced by clinical isolates of pseudomonas aeruginosa and detection of chia responsible gene. International Journal of Research in Pharmaceutical Sciences.2020; 11 (2):1318-1330. doi: 10.26452/ijrps.v11i2.1989.

49. *Farman MS, Akoul MA.* The Effect of Toxoplasmosis on Pregnant Women and Its Diagnosis by "VIDAS Test of IgG Avidity". Asian Journal of Water, Environment and Pollution. 2022; 19(3):75-8. doi: 10.3233/AJW220042.

50. *Hamoode RH, Alkubaisy SA, Sattar DA, Hamzah SS, Saleh TH, Al-Rubaii BA.* Detection of anti-testicular antibodies among infertile males using indirect immunofluorescent technique. Biomedicine. 2022;42(5):978-982. doi: 10.51248/.v42i5.1963.

51. *Mohsin MR, Al-Rubaii BA.* Bacterial growth and antibiotic sensitivity of Proteus mirabilis treated with anti-inflammatory and painkiller drugs. Biomedicine. 2023;43(2):728-34. doi: 10.51248/.v43i02.2693.