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Association of the IL1RN gene VNTR polymorphism (rs2234663) with chronic inflammation-associated cancer

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One of the main tasks of modern medicine is to identify genetic predisposition to common diseases using molecular markers. This plays a crucial role in enabling early diagnosis and timely prevention. Currently, diseases that are either hereditary or non-hereditary, and which are caused by endogenous and exogenous factors, mutagenesis, and acute or chronic inflammatory processes, are the leading cause of both incidence and mortality. Of particular importance among these are various forms of cancer, which are associated not only with genetic factors but also with chronic inflammation. It is well established that proinflammatory cytokines, their biosynthesis and the proper functioning of signalling pathway components play a key role in the development and regulation of inflammatory processes, particularly chronic ones. In this context, along with agonists of interleukin-1 (IL-1), the interleukin-1 receptor antagonist (IL-1RA) and the gene encoding it (IL1RN) are critically involved in modulating IL-1 activity. The aim of the present study was to determine the association between the VNTR polymorphism (rs2234663) located in the second intron of the IL1RN gene and the risk of cancer presumably associated with chronic inflammation. The study material consisted of genomic DNA isolated from peripheral blood samples of cancer patients (experimental group, EG, n=80) and conditionally healthy individuals (control group, CG, n=84). Genotyping of the IL1RN VNTR polymorphism (rs2234663) was performed using the polymerase chain reaction (PCR) method with specific primers. Allele and genotype frequencies were calculated for both groups. Although all known alleles of the IL1RN gene were detected in the studied cohorts, several genotypes (*2*5, *2*6, *3*4, *3*5, *3*6, *4*5, *4*6, and *5*6) were not observed in either group. In the experimental group, the frequency of the normal allele *1 was approximately 1.4-fold lower, whereas the frequency of the mutant allele *2 was about 1.6-fold higher compared with the control group. Overall, the homozygous mutant genotype (*2*2) occurred approximately 2.1 times more frequently in cancer patients than in controls. To evaluate the strength of association between the IL1RN polymorphism and cancer susceptibility, odds ratios (OR), relative risks (RR), 95% confidence intervals (CI), Z-test statistics, and corresponding P values were calculated. The association between the risk allele *2 and cancer predisposition was statistically significant (OR≈2.2, RR≈1.53, P≈0.001). A pronounced association was also observed for the homozygous genotype *2*2 (OR≈2.84, RR≈2.18, P≈0.004). Notably, compared with heterozygous carriers (*1*2), individuals homozygous for the *2 allele (*2*2) exhibited approximately 2.4-fold higher odds (OR*2*2/OR*1*2) and about 2.0-fold higher relative risk (RR*2*2/RR*1*2) of developing cancer associated with chronic inflammation. Of the analysed genetic models, only the dominant model (*2*2 vs. *1*1 + *1*2) showed a statistically significant association with cancer risk (OR≈2.97, RR≈2.10, P=0.003).

***Keywords:** Inflammation, cytokine, interleukin-1, agonist, antagonist, risk allele, cancer, association*

INTRODUCTION

A continuous rise is currently being observed in the incidence of both hereditary and non-hereditary cancers, which are linked to ongoing mutagenic processes in the human body. This rise is occurring alongside an increase in autoimmune, allergic and inflammatory diseases. Analysis of global epidemiological data shows that around six in ten disease-related deaths are due to chronic inflammatory conditions, such as stroke, chronic cardiovascular and respiratory diseases, allergies, various types of cancer, obesity, diabetes and related disorders (Pahwa et al., 2022; WHO Statistics, 2024). Cancer, particularly inflammation- and infection-associated malignancies, represents a growing global health burden, with steadily increasing incidence and mortality rates. Contemporary global cancer incidence and mortality statistics (Worldwide Cancer Data, 2024; Siegel et al., 2025) provide a comprehensive overview of these trends, as do reports from the American Cancer Association, which provides one of the most systematic and representative cancer surveillance datasets worldwide (Global Cancer Statistics, 2024).

The concept of linking malignant cellular transformation to chronic inflammatory diseases was first proposed in the nineteenth century. Since then, epidemiological evidence has demonstrated that inflammatory processes arising from various types of tissue injury, physical, chemical and biological, contribute to the development of at least 15% of all cancer types. The association between chronic inflammation and cancer is particularly evident in gastrointestinal diseases such as chronic hepatitis B and C, B+C co-infections, Barrett's oesophagus, gastric infections, chronic pancreatitis, ulcerative colitis and Crohn's disease. Similar relationships are also evident in the respiratory system, for example, in asbestosis, tuberculosis, chronic bronchitis, and pneumonias caused by exposure to wood and fur dust, as well as by airborne pathogens (e.g. coronaviruses). Furthermore, chronic inflammatory conditions of the genitourinary system, including chronic cervicitis and prostatitis, as well as sexually transmitted infections, exemplify the close interplay between persistent inflammation and carcinogenesis (Furukawa et al., 2011; Brovkina et al., 2022; Dinarello, 2023; Albini et al., 2025; Lee et al., 2025; Wu et al., 2025; Xu et al., 2025).

The molecular mechanisms underlying the bidirectional interplay between carcinogenesis and inflammation are based, firstly, on the expression of receptors for cytokines, chemokines, growth factors and immunoregulatory molecules by normal epithelial cells and, secondly, on the constitutive expression and activation-induced secretion of cytokines, eicosanoids, endothelins, defensins, nitric oxide and mediators of cell–cell interactions by these same cells. Elucidating the role of the highly complex, multifunctional and multicomponent immune system, which encompasses both innate and adaptive immunity and is governed by sophisticated regulatory mechanisms, is critical in this context. Specifically, pathogenic stimuli in the cytosol give rise to pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), or lifestyle-associated molecular patterns (LAMPs). These patterns collectively trigger the assembly of multiprotein complexes known as inflammasomes. These pattern-recognition receptor-based platforms are a key part of the inflammatory immune response, ultimately leading to the activation of pro-inflammatory cytokines, including interleukins such as IL-1 and IL-18, as well as TNF and IFNs. The resulting cytokine cascade drives the induction and execution of inflammation-associated immune responses (Weber et al., 2010; Gong et al., 2020; Zindel & Kubes, 2020; Liu et al., 2022; Tokarz-Deptuła et al., 2024; Gartland et al., 2025).

Disruptions to this tightly regulated process, which occurs at any level, may lead to chronic and systemic inflammation. This inflammation can then promote the emergence of cancer stem cells. The dysregulation of signalling pathways that mediate the activity of pro-inflammatory and inflammatory cytokines, which are secreted by immune cells involved in inflammatory responses and their resolution, including natural killer (NK) cells, T helper cells, macrophages and related immune cell populations, can facilitate tumour initiation and progression. Once established, cancer stem cells may evade recognition by NK cells of the immune system and undergo unchecked expansion. Within this regulatory network, the pro-inflammatory cytokine interleukin-1 (IL-1) and its receptor (IL-1R) play a pivotal role (Weber et al., 2010). The IL-1 signalling axis comprises two endogenous agonists (IL-1α and IL-1β) and a naturally occurring antagonist (IL-1RA). Genetic alterations affecting these components, including VNTR-type polymorphisms in the IL1RN gene that encodes IL-1RA, particularly the IL1RN*2 mutant allele, can disrupt the normal function of the IL-1 signalling pathway. This pathway is a critical mediator of immune responses and its disruption can contribute to the development of a range of diseases, including multiple cancer types (Jaiswal et al., 2012; Sousa et al., 2013; Hashemi et al., 2015; Nedumpun et al., 2017; Saad et al., 2020).

Comprehensive insights into the relationship between tumour development and inflammation, particularly about the involvement of reactive oxygen, nitrogen and sulphur species (ROS, RNS and RSS) in this process, as well as the role of other key molecular and cellular factors, can be found in the works of F.R.Greten and S.I.Grivennikov (2019) and F.Okada et al. (2021). Additionally, H.Zhao et al. (2021) have provided generalised schematic representations of the signalling pathways involved in inflammation-driven tumourigenesis, together with their principal components (including proteins and immune cell populations).

A multiprotein oligomeric complex known as the inflammasome initiates the cascade of inflammatory responses. The activation of inflammasomes promotes the maturation and secretion of the pro-inflammatory cytokines IL-1β and IL-18. These cytokines then trigger pyroptosis, which is a distinct form of programmed cell death. It is widely hypothesised that inflammasome dysregulation is one of the fundamental mechanisms underlying the development of numerous inflammation-associated diseases. Several key studies (Broz and Dixit, 2016; Jin and Yin, 2019; C.Zhao and W.Zhao, 2020; Seok et al., 2021; Inflammasomes, 2022) provide detailed descriptions of canonical and non-canonical inflammasomes, as well as their therapeutic regulatory roles in inflammatory processes.

The interleukin-1 (IL-1) family is a group of proteins that play central roles in both innate and adaptive immune responses, some of which are pro-inflammatory and some of which are anti-inflammatory. The most extensively studied and biologically significant member of this family is IL-1β. As a key mediator of inflammation, IL-1β induces fever and promotes immune activation by binding to IL-1 receptor type 1 (IL-1R1). Its production and secretion are tightly regulated and dependent on inflammatory stimuli. Initially, transcription of the biologically inactive precursor form, pro-IL-1β, is induced by the activation of Toll-like receptors (TLRs), tumour necrosis factor (TNF) signaling, or the engagement of IL-1 receptors by mature IL-1α or IL-1β (Yazdi and Ghoreschi, 2016).

Only upon the activation of caspase-1 are both IL-1 receptors (IL-1R1 and IL-1R2) released from their associated cytokines (IL-1β and IL-1α). This allows the conversion of the precursor cytokines into their mature forms, which are then secreted. Interestingly, IL-1R2 appears to regulate IL-1α activation differently during necrotic cell death, independently of inflammasome signalling. In this context, the IL-1 receptor antagonist (IL-1RA) acts as a natural inhibitor of IL-1 receptor-mediated signalling (Yazdi and Ghoreschi, 2016; Fields et al., 2019).

Cellular senescence acts as a critical barrier against oncogenic transformation. It is characterised by irreversible cell-cycle arrest and elevated levels of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-α. The IL-1 cytokine family comprises 11 members that play a key role in regulating inflammation, including IL-1α, IL-1β, IL-1RA, IL-18, IL-33, IL-36RA, IL-36α, IL-36β, IL-36γ, IL-37 and IL-38.

Signaling cascades initiated by IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β and IL-36γ converge on the activation of the MAPK and NF-κB pathways. This ultimately leads to the transcriptional upregulation of pro-inflammatory cytokines, chemokines and secondary mediators of inflammation. Furthermore, mounting evidence suggests that various IL-1 family members play a role in regulating T-helper cell differentiation and effector functions.

Several members of the interleukin-1 (IL-1) family function as endogenous antagonists of IL-1 and IL-36 signaling, thereby exerting potent anti-inflammatory effects. The IL-1 receptor antagonist protein (IL-1RA) negatively regulates IL-1 signaling by competitively binding to IL-1 receptor type 1 (IL-1R1), thus preventing its interaction with the pro-inflammatory agonists IL-1α and IL-1β. Analogously, IL-36 receptor antagonist (IL-36RA) binds to IL-1 receptor–related protein 2 (IL-1Rrp2) and inhibits IL-36-mediated signal transduction. In addition, it has been proposed that IL-37 (IL-1F7) and IL-38 (IL-1F10), either individually or as part of regulatory complexes, also exert anti-inflammatory and immunosuppressive functions (for a comprehensive review, see: *IL-1 (Interleukin-1) Family*, 2025).

IL-1RA is a key anti-inflammatory regulator within the IL-1 cytokine family. It modulates the biological activity of IL-1 cytokines, thereby limiting their pro-inflammatory potential. It is produced by a variety of cell types, particularly macrophages, monocytes and epithelial cells. It is encoded by the IL1RN gene located on chromosome 2q14.1 and is also known by several alternative names, including DIRA, ICIL-1RA, IL-1RN, IL-1ra, IL-1ra3, IL1F3, IL1RA, IRAP and MVCD4.

IL-1RA was first isolated from human leukaemia monocyte cell lines (THP-1 cells) by Bienkowski et al. in 1990. This protein shares approximately 30% amino acid sequence homology with IL-1β, and can bind to IL-1 receptors even when there is no overt cellular activation. In vitro, IL-1RA effectively blocks IL-1-mediated stimulation of thymocytes (T lymphocytes), fibroblasts, endothelial cells and osteogenic cells, while in vivo it acts as a potent inhibitor of IL-1-driven inflammatory responses.

The gene encoding the interleukin-1 receptor antagonist protein (IL-1RA) was first cloned and characterised in terms of its chromosomal localisation and expression by D. B. Carter and colleagues in 1990, a discovery that was followed shortly thereafter by that of A. C. Lennard and colleagues in 1992. A comprehensive and critical review of the biology, regulation and functional relevance of IL-1RA was later published by A.C.Lennard (2017). Detailed genomic and functional information on the IL1RN gene is available through public databases, including the NCBI Gene repository (Gene ID: 3557; https://www.ncbi.nlm.nih.gov/gene/3557).

A wide range of studies have addressed the association between the variable number of tandem repeats (VNTR) polymorphism of the IL1RN gene and susceptibility to inflammatory and inflammation-related diseases. These studies have been reported in both review articles and original research publications, and are discussed in the relevant sections of this manuscript. Our previous studies investigated the links between the A→G single nucleotide polymorphism (SNP) (2758 A>G, rs696) of the NFKBIA (or NF-κBIA) gene and diabetes mellitus and cancer development (Akhundova et al., 2022), and between the VNTR polymorphism (rs223466) of the IL1RN gene and susceptibility to and severity of coronavirus infection (Naghiyeva et al., 2023).

The primary objective of this study is therefore to evaluate the association between the VNTR polymorphism of the IL1RN gene (rs223466) and inflammation-driven carcinogenesis.

MATERIALS AND METHODS

The material of the study: The main material of the study was DNA isolated from blood samples collected from unrelated patients of different ages, diagnosed with various forms of cancer (experimental group (EG) - 80 people (44 women, 36 men) and conditionally healthy individuals (control group (CG) - 84 people (47 women, 37 men)) working in different occupational fields. Samples were collected on a voluntary basis in accordance with the Helsinki Declaration and international bioethical norms. Prior to the collection of samples, a letter was sent to the Oncology Clinic of the Azerbaijan Medical University, Ministry of Health of the Republic of Azerbaijan, and official consent was obtained.

It should be noted that the age range of patients in the studied EG was wide, from 45 (1980) to 73 (1952). The age range of the conditionally healthy control samples (CG) was from 43 (1982) to 75 (1950). Blood samples and information about the patients and the course of the disease were provided by the hospital where the patients in the experimental group (EG) were treated. According to the study, patients with stages II (33 people), III (40 people) and IV (seven people receiving intensive chemotherapy) of cancer were involved. The study also involved conditionally healthy individuals who did not exhibit any symptoms or complaints relating to the disease under study during the study period.

DNA isolation: The DNA from collected blood samples (200 l) was extracted with a reagent kit, "DiatomTMDNA Prep 200" (Isogen, Moscow, RF), based on the manufacturer's protocol. The amount of DNA isolated and the degree of purity were measured with a NanoDrop 2000 spectrophotometer. The isolated DNA was stored at -20°C (or for a long time at -80°C).

Detection of rs2234663 polymorphism of IL1RN gene: The IL1RN gene rs223466 polymorphism was determined by polymerase chain reaction (PCR) using pairs of specific primers: Forward: 5' CTCAGCAACACTCCTAT-3'; Reverse: 5' TCCTGGTCTGCAGGTAA-3' (Arnalich et al., 2022; Kayar et al., 2015; Saad et al., 202; Swellam et al., 2013). The PCR conditions were determined by the gradient PCR. The gradient PCR was performed at 12 temperatures in the range of 45-60ºC, divided equally, and 48ºC was taken as the best annealing temperature of the primers. PCR reaction conditions were as follows: initial denaturation at 95ºC for 5 min before the first cycle; 60 sec at 94ºC (denaturation), 60 sec at 48ºC (annealing), 60 sec at 72ºC (elongation) - 35 cycles; and final elongation - 10 min. The synthesized fragments were electrophoresed in a 1.8% agarose gel using a 100 b.p. ladder (MBI Fermentas), visualized using ethidium bromide staining and documented by UVITES Gel Documentation System (CS Ltd. UK).

As a result of PCR, the following fragments were synthesized:

IL-1RN1 (allele *1), R=4, 410 b.p. fragment;

IL-1RN2 (allele *2), R=2, 240 b.p. fragment;

IL-1RN3 (allele *3), R=3, 325 b.p. fragment;

IL-1RN4 (allele *4), R=5, 500 b.p. fragment;

IL-1RN5 (allele *5), R=6, 585 b.p. fragment;

IL-1RN6 (allele *6), R=1, 155 b.p. fragment.

Statistical analyses: The obtained results were statistically analyzed using the Microsoft Excel software. Odds ratio (OR), relative risk (RR), etc., parameters of alleles, genotypes and genetic models associated with susceptibility to infection/course of the disease were calculated using online calculators [https://www.medcalc.org/calc/; http://vassarstats.net/odds2x2.html].

RESULTS AND DISCUSSION

A total of 164 people were included in the study, and genetic profiles were obtained for 164 of them (73 men and 91 women). The following genotypes were observed by gender in the experimental and control groups (Table 1). It should be noted that no statistically significant associations were observed between the *2 allele or the genotypes involving this allele and disease susceptibility with respect to sex or age (in both cases $p>0.05$).

Some of the detected genotypes are shown as an example in the Figure, and the frequencies of alleles and genotypes are given in Table 2.

In summary, the 4-repeat (410 bp, IL1RN*1 – normal allele (*1)) and 2-repeat (240 bp, IL1RN*2 – major variant allele (*2)) alleles of the gene are more common (~90%), while the frequency of other alleles is generally ~10%.

Numerous studies have investigated the association between various mutations of the IL1RN gene, which encodes the interleukin-1 receptor antagonist (IL-1RA), as well as its variable number tandem repeat (VNTR) polymorphism (rs2234663) located in intron 2 and consisting of ~86 bp repeats, and a broad spectrum of inflammatory and inflammation-related diseases.

Table 1. Distribution of genotypes detected in the study groups (EG and CG) by gender

Genotypes	Experimental group			Control group		
	Total	Women	Men	Total	Women	Men
*1*1	20	10	10	31	17	14
*1*2	17	10	7	22	13	9
*1*3	2	1	1	4	2	2
*1*4	1	0	1	2	1	1
*1*5	3	2	1	3	2	1
*1*6	1	1	0	2	1	1
*2*2	29	16	13	14	8	6
*2*3	2	2	0	2	1	1
*2*4	3	2	1	2	1	1
*5*5	2	0	2	2	1	1

Table 2. The amounts and frequencies of alleles/genotypes detected in the experimental and control groups

GROUPS	Alleles (amount (%))					
	*1	*2	*3	*4	*5	*6
Experimental group (cancer patients with various diagnoses, n=80)	64 (40.0%)	80 (50.0%)	4 (2.5%)	4 (2.5%)	7 (4.4%)	1 (0.6)
Control group (conditionally healthy individuals, n=84)	95 (56.5%)	54 (32.1%)	6 (3.6%)	4 (2.4%)	7 (4.2%)	2 (1.2%)
By population (n=164)	159 (48.5%)	134 (40.9%)	10 (3.0%)	8 (2.4%)	14 (4.3%)	3 (0.9%)

Genotypes (amount (%))									
*1*1	*1*2	*1*3	*1*4	*1*5	*1*6	*2*2	*2*3	*2*4	*5*5
Experimental group (cancer patients)									
20 (25.00)	17 (21.25)	2 (2.50)	1 (1.25)	3 (3.75)	1 (1.25)	29 (36.25)	2 (2.50)	3 (3.75)	2 (2.50)

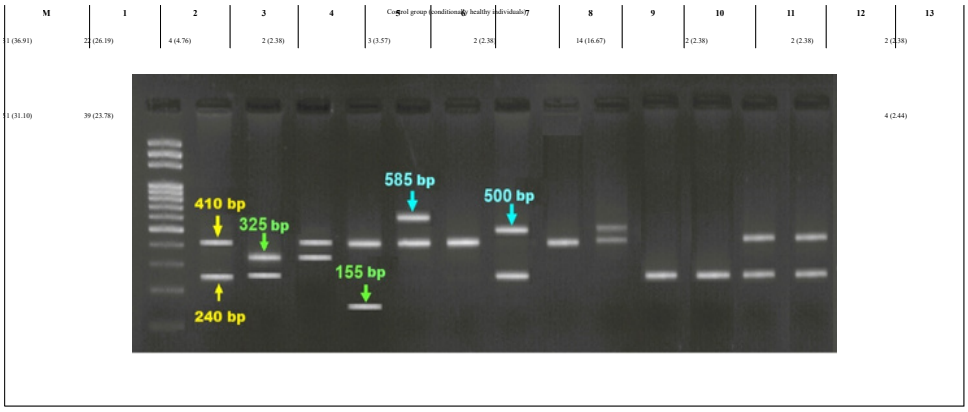

Fig. Electrophoresis of samples belonging to individuals in the experimental (samples 2, 3, 5, 7, 9, 11 and 13) and control (samples 1, 4, 6, 8, 10 and 12) groups.

M – 100 bp ladder.

These include, but are not limited to, osteoarthritis, type 1 diabetes mellitus in children, type 2 diabetes mellitus, thrombocytopenia, ischemic stroke, hepatitis E virus infection, endometriosis, childhood melanoma, breast cancer, skin cancer, gastric cancer, and colorectal cancer (Attur et al., 2020; Bent et al., 2018; Broer et al., 2017; Clark et al., 2017; Dorling et al., 2022; El-Serag et al., 2006; Ibrahimi et al., 2022; Mier-Cabrera et al., 2022; Pesmatzoglou et al., 2012; Tripathy et al., 2024; Yang et al., 2018; see also references in Table 3).

In most of these studies, the 2 allele, which is a tandem repeat approximately 240 bp in length, is reported as a potential risk allele. Furthermore, several genotypes involving this allele have been associated with an increased risk of disease. However, contradictory findings have also been reported, which are most likely due to differences in allele frequencies and the strength of associations between inflammation-driven diseases and the IL1RN alleles and genotypes being investigated, which vary between populations.

The distribution and frequency of the two most prevalent IL1RN alleles (*1 and *2) across different populations is of particular interest overall. Table 3 summarises the frequencies of these alleles in selected populations, based on data derived from population-based studies conducted worldwide.

As shown in Table 3, the frequency of the *2 allele varies considerably between populations. In East and Southeast Asian populations (including most regions of China and Korea, except the Han population), African populations (e.g., Sudan) and certain Russian populations, the frequency of the *2 allele is below 10%.

Table 3. The IL1RN gene *1 and *2 alleles frequencies in different populations, %.

No	Population	Population size (n) and/or studied disease	*1 allele	*2 allele	References
1	Azerbaijan*	>460 (~240 healthy, ~220 unhealthy)	62.1	30.3	Naghiyeva et al., 2023; Mustafayev et al., 2025
2	UK (United Kingdom)	70	73.6	21.4	Tarlow et al., 1993
3	China, 19 population	1352	91.3	6.4	Jiang et al., 2010
4	China, Han	256	83.0	16.2	Xu et al., 2011
5	China, She	252	93.3	6.7	Xu et al., 2011
6	Italy	515 (382 healthy, 133 cutaneous melanoma)	73.3	24.0	Cauci et al., 2019
7	USA, 3 population	Total 782 (480 cases of ischemic stroke and 302 controls	76.5	24.9	Worral et al., 2007
8	USA (N.Jersey and North Carolina, Caucasians)	Total 896 (516 ischemic stroke and 380 control)	67.8	27.1	Peddareddygari et al., 2014
9	Türkiye	198 (94 rheumatoid arthritis, 104 control)	76.6	20.7	Arman et al., 2006
10	Türkiye	133 (33 cancer, 100 healthy)	51.5	44.7	Gümüşay et al., 2019
11	Korea	640	91.7	6.0	Um and Kim, 2003
12	Iran	515 (265 pulmonary tuberculosis and 250 healthy)	~81.0	~15.0	Hashemi et al., 2015
13	Iran	223 (126 cancer, 97healthy control)	77.4	17.0	Abbasian et al., 2018
14	Iran	275 (123 CRC, 152 control)	58.9	35.6	İbrahimi et al., 2019
15	Iran	356 (120 NHL, 50 HL, 186 control)	51.4	30.5	Sarani et al., 2021
16	India	336 (119 recurrent pregnancy loss and 200 healthy control women)	62.5	35.3	Nair et al., 2014
17	India	190 (86 kidney damage, 104 normal kidney function)	82.4	17.6	Bhaskar and Pattnaik, 2023
18	India	689 (331 male infertility, 358 healthy fertile men)	58.6	40.8	Jaiswal et al., 2012
19	Northeast Brazil	153 (39 osteomyelitis, 114 healthy)	84.3	15.00	Alves de Souza et al., 2017
20	Mexica	630 (frailty syndrome: prefrail 237, frail 72 and non-frail 319)	80.0-87.5	12.5-20.0	Pérez-Suáre et al., 2016

21	Mexica	486 (230 with CRC and 256 healthy)	54.5	41.8	Gallegos-Arreola et al., 2024
22	Poland	90 (from among 402 premature infants)	68.9	29.4	Szpecht et al., 2020
23	Poland	795 (366 gastric cancer cases and 429 controls)	57.0	33.6	El-Omar et al., 2000
24	Portugal	58	48.3	10.3	Sampaio-Fernandesa et al., 2015
25	Portugal	545 (112 patients with the NPS and 433 healthy)	66.2	31.7	Sousa et al., 2013
26	Egypt	200 (120 T1DM, 80 control)	71.0	23.0	Abed et al., 2022
27	Egypt	140 (80 ASD, 60 control)	70.4	29.3	Saad et al., 2020
28	Egypt	185 RA pacients	65.8	34.2	Swellam et al., 2013
29	Russia	196	72.2	9.9	Udina et al., 2022
30	Africa, Sudan	114 *54 cancer, 60 healthy)	89.3	9.4	Abeer et al., 2019
31	Sweden	259 (125 CC, 134 control)	68.5	28.8	Viet et al., 2005

Note: *In many cases, allele frequencies were calculated by us based on data provided in references.*

By contrast, most populations in South Asia (India), Europe, the Caucasus and the Middle East, including the Azerbaijani population, have allele frequencies ranging from approximately 10% to 35%. Interestingly, in populations from Turkey, India and Mexico, the frequency of the *2 allele exceeds 40%.

The Azerbaijani population exhibits an intermediate distribution comparable to that of neighbouring regions. Interestingly, a high frequency of the 2 allele is often seen in groups of people with diseases, which further supports its potential role as a genetic risk factor.

The VNTR polymorphism (rs2234663) under investigation has also been examined in the context of SARS-CoV-2 infection and disease progression (Naghiyeva et al., 2023). In that study, a non-specific allele of approximately 1,100 bp was reported in two cases, but this was not detected in our experimental cohort. Conversely, the smallest allele described in the previous study, at around 155 bp, was not present in their analysis, but was detected in our study, in both the experimental group (one case) and the control group (two cases). This discrepancy is likely due to the larger sample size used in the present study.

Analysis of the literature indicates that the combined frequencies of the normal (*1) and more common mutant (*2) alleles of the IL1RN gene account for approximately 90–93% of all alleles. The remaining rare (*3, *4, *5 and *6) alleles collectively comprise ~5–7%. In both the experimental (EG) and control (CG) groups, as well as in the overall population sample, there was no significant difference in the cumulative frequencies of alleles 1 and 2, which represent the predominant fraction of total alleles (~90% in EG, ~89% in CG and a similar proportion in the population sample).

In our study, a slight decrease in the frequencies of the major alleles (*1 and *2) and a modest increase in the minor alleles (*3, *4, *5, and *6) (~3–4% each) were observed. Due to the low prevalence of the minor alleles and the difficulty of establishing statistically significant correlations or associations, subsequent analyses and interpretations focused primarily on alleles *1 and *2 and the genotypes they form. Notably, the frequency of the *1 allele was approximately 1.4-fold lower and the frequency of the *2 allele was approximately 1.6-fold higher in the experimental group than in the control group.

The distribution of genotypes observed in the study groups exhibited some notable differences. Certain genotypes, including *2*5, *2*6, *3*4, *3*5, *3*6, *4*5, *4*6, and *5*6 were not detected in either the experimental (EG) or control (CG) groups. Interestingly, the genotypes *1*6 and *2*3 were observed exclusively in the EG, and carriers of

these genotypes represented the youngest cancer patients in terms of age. Furthermore, in the total cohort (EG), the *1*4 and *5*5 genotypes were absent among female patients, whereas the *1*6 and *2*3 genotypes were not observed among male patients.

There were pronounced differences in the frequencies of the homozygous *2*2 genotype between groups. Overall, the prevalence of this genotype, which is associated with an increased risk, was higher than in our previous study (Naghiyeva et al., 2023): ~26.22% vs. 11.2%. Notably, the frequency of the *2*2 genotype in the experimental group (EG) was approximately 2.1-fold higher (36.25%) than in the control group (CG) (16.67%).

The frequencies of the homozygous *1*1 and heterozygous *1*2 genotypes were as follows: *1*1 25.00% in EG, 36.91% in CG, and 21.25% in the overall studied population; *1*2: 21.25% in EG, 26.19% in CG, and 23.78% in the overall population. Accordingly, the frequency of the *1*1 genotype in EG was approximately 1.5-fold lower, while that of the *1*2 genotype was approximately 1.2-fold lower compared to CG.

To evaluate the association between cancer susceptibility and the presence of the mutant *2 allele of the IL1RN gene, which encodes the natural antagonist of the pro-inflammatory cytokine IL-1 receptor, statistical parameters including odds ratios (ORs) and relative risks (RRs) with 95% confidence intervals (CIs), Z-test statistics reflecting the distribution coefficient, and significance levels (P) were calculated for the study groups and the overall population using online calculators (https://www.medcalc.org/calc/; http://vassarstats.net/odds2x2.html).

Calculations were performed relative to the control group, and only genotypes carrying the mutant *2 allele were included in the analysis. The results of the statistical evaluation of the risk allele (*2) and the genotypes formed with its participation (*2*1 and *2*2) in the studied groups are summarized in Table 4.

As shown in Table 4, the odds ratio (OR = 2.1991) and relative risk (RR = 1.5329) for the association between the *2 risk allele and cancer are both greater than one and statistically significant ($P < 0.001$), indicating that this allele meaningfully contributes to cancer susceptibility. A different pattern was observed for genotypes containing the *2 allele. Although the odds ratio (OR=1.1977) and relative risk (RR=1.1069) for the heterozygous *1*2 genotype were slightly greater than unity, no statistically significant association with cancer was detected (P=0.419).

Table 4. Statistical data of the risk allele (*2) and genotypes (*1*2 and *2*2) detected in the studied groups.

Allee/Genotype	Odds ratio (OR, CI=95%)	Relative risk (RR)	P-value	Z-statistics
Allelles				
*1	-	-	-	-
*2	2.1991 (1.3763-3.5137)	1.5329 (1.1840-1.9846)	0.001	1.96
Genotypes				
*1*1	-	-	-	-
*1*2	1.1977 (0.5137-2.7925)	1.1069 (0.6893-1.7774)	0.419	0.418
*2*2	2.8431 (1.3662-5.9166)	2.1750 (1.2429-3.8061)	0.0036	2.795

Table 5. Results of statistical analysis of genetic models in the studied group.

Genetic models	Odds ratio (OR, CI=95%)	Relative risk (RR)	P-value	Z-statistics
Dominant ***2*2 vs (*1*1+*1*2)**	2.97 (1.38-6.37)	2.10 (1.23-3.61)	0.003	2.70
Recessive **(*2*2+*1*2) vs *1*1**	1.98 (0.97-4.03)	1.30 (0.99-1.70)	0.062	1.87
Overdominant **(*1*1+*2*2) vs *1*2**	1.41 (0.66-2.99)	1.11 (0.89-1.38)	0.240	0.894

This suggests that the normal *1 allele may offset the harmful effects of the *2 allele, thereby reducing the risk of inflammation-associated cancer. In contrast, the homozygous *2*2 genotype demonstrated a pronounced and statistically significant association with cancer. In contrast, the homozygous *2*2 genotype demonstrated a pronounced and statistically significant association with cancer. The corresponding values (OR=2.8431, RR=2.1750, P=0.0036) indicate a strong correlation between this genotype and disease susceptibility. Specifically, compared with heterozygous carriers (*1*2), individuals homozygous for the *2 allele (*2*2) exhibited approximately a 2.4-fold increase in odds (OR∗2∗2:OR∗1∗2) and about a 2.0-fold increase in relative risk (RR∗2∗2:RR∗1∗2) of developing cancer associated with chronic inflammation.

In this context, it is particularly interesting to analyse the associations between the possible genetic models formed by the *1 and *2 alleles and cancer that is presumed to develop as a result of chronic inflammation. This approach also allows us to indirectly assess the potential functional capacity of the immune system, depending on the presence of the mutant risk allele, which can lead to acute and severe chronic inflammatory responses. As shown in Table 5, a statistically significant association with cancer was detected only for the dominant genetic model (P=0.003). This finding is fully consistent with the results obtained for the individual analysis of the *2 risk allele and the homozygous *2*2 genotype, both of which demonstrated a significant association with cancer presumably induced by chronic (acute and severe) inflammation (Table 4).

Let us consider the genotype ratios corresponding to the dominant, recessive, and overdominant (codominant) genetic models formed by the presence of the *2 risk allele, analyzed separately within each study group (experimental group, EG; control group, CG) as well as in the total population sample. Under the dominant model of the *2 allele, the genotype ratios were as follows: in the experimental group, *2*2/(*1*1+ *1*2)=0.78; in the control group, *2*2/(*1*1+*1*2)=0.26; and in the overall population sample, *2*2/(*1*1+*1*2)=0.48. These values suggest that the association between the dominance of the *2 risk allele and cancer (inflammation-related pathology) is approximately 3.0 times higher in the experimental group than in the control group, and around 1.6 times higher in the entire population.

Assuming a recessive effect of the *2 allele in the genetic model, the genotype ratios were as follows: in the experimental group, (*2*2+*1*2)/*1*1=2.30; in the control group, (*2*2+*1*2)/*1*1=1.17; and in the overall population sample, (*2*2+*1*2)/*1*1=1.61. Thus, although the presumed association between the *2 allele and inflammation-related cancer under the recessive model is less pronounced than that observed in the dominant model, a measurable disease risk remains. Specifically, this ratio is approximately 2.0-fold higher in the experimental group than in the control group and about 1.4-fold higher than in the population sample as a whole.

According to the overdominant (codominant) genetic model, the ratio of homozygous genotypes (*2/*2+*1/*1) to the heterozygous genotype (*1/*2) was 2.88 in the experimental group, 2.05 in the control group, and 2.41 in the overall population. Within this model, the ratio reflecting the presumed association between the presence of the *2 allele and cancer (inflammation-related pathology) was modestly elevated in the experimental group. In quantitative terms, this indicator was approximately 1.4-fold higher in the experimental group compared with the control group and about 1.2-fold higher than that observed in the population sample overall.

A comparative analysis of genotype ratios across all three genetic models demonstrates that, despite the relatively lower numerical values observed in the dominant model involving only the homozygous *2*2 genotype, the effect of this allele/genotype on disease susceptibility and progression is substantial. This observation is fully consistent with the results obtained for the corresponding alleles and genotypes in Table 4, thereby reinforcing the evidence for the significant contribution of the *2 allele to cancer risk, presumably mediated by chronic inflammation.

CONCLUSION

In order to elucidate the potential genetic associations involved in the development of cancer, the VNTR-type polymorphism (rs2234663) of the IL1RN gene was investigated. This gene encodes the interleukin-1 receptor antagonist (IL-1RA), which is a key regulator of the pro-inflammatory cytokine interleukin-1. The study population comprised a representative sample of 164 individuals from the Azerbaijani population. The study population comprised an experimental group (EG) of patients diagnosed with various forms of cancer and a control group (CG) of individuals in good health. The frequencies of all known alleles and genotypes of the IL1RN gene were determined within the framework of this study.

According to the results obtained, the frequency of the common *1 allele, reported as the most prevalent in many populations worldwide, was 40.0% in the EG and 56.5% in the CG. Conversely, the frequency of the *2 allele, considered a potential risk allele, was notably higher among cancer patients (50.0%) than in the control group (32.0%).

A comparative analysis of the observed allele frequencies with published data from 30 populations across different geographical regions revealed that the frequency of the *2 allele in the Azerbaijani population is relatively high, comparable to that reported for Caucasian, Middle Eastern and certain American populations. Notably, numerous studies have documented an increased prevalence of the *2 allele in cohorts affected by disease, further supporting its proposed role as a genetic risk factor in the development of cancer and other inflammation-associated pathologies.

Association analyses of the IL1RN*2 risk allele, genotypes containing this allele (i.e., *1*2 and *2*2), and the corresponding genetic models demonstrated that the *2 allele is significantly associated with cancer that is presumed to develop as a result of chronic inflammation. Notably, the odds ratio (OR ≈ 2.2) and relative risk (RR ≈ 1.53) for the association between the *2 allele and cancer were greater than one and statistically significant (P ≈ 0.001), suggesting a substantial role for this allele in disease susceptibility.

Stronger association parameters were observed for the homozygous *2*2 genotype (OR ≈ 2.84, RR ≈ 2.18, P ≈ 0.004), suggesting the presence of an allele-dose effect. Further comparative analysis showed that individuals homozygous for the *2 allele had approximately 2.4 times the odds and 2.0 times the relative risk of developing cancer associated with chronic inflammation compared to heterozygous carriers (*1*2).

Of the evaluated genetic models, only the dominant model (*2*2 vs. *1*1 + *1*2) showed a statistically significant association with cancer risk (OR ≈ 2.97; RR ≈ 2.10; P = 0.003), which is consistent with the allele- and genotype-based analyses.

Taken together, the results obtained indicate a potential association between the IL1RN gene *2 allele and genetic susceptibility to cancer and other inflammation-related diseases. However, before considering this allele as a reliable clinical prognostic marker, further large-scale, long-term studies across diverse populations are required. The relatively high frequency of the *2 allele in the population (>30%) underscores the importance of evaluating its role in predisposing individuals to inflammatory diseases. Nevertheless, therapeutic interventions targeting the IL-1 signalling pathway, such as the use of the recombinant interleukin-1 receptor antagonist anakinra (Kineret®), should only be considered within established clinical indications and under appropriate medical supervision.

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AUTHOR CONTRIBUTION STATEMENT

Nurmammad Mustafayev: scientific idea, conceptualization, study design, experimental procedures, discussion, and writing of the first draft.

Lala Akhundova, Shalala Majidova, and Nigar Mammadli: sample collection, DNA isolation, genotyping, statistical analysis, and other experimental procedures.

Ahliman Amiraslanov and Irada Huseynova: discussion, reviewing, and editing of the manuscript.

Ahliman Amiraslanov: expert recommendations and comments related to cancer disease.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to the publication of this manuscript.

ETHICAL ISSUES

The research followed the ethical principles of the Declaration of Helsinki. This study was approved by the ethics committees of the Institute of Molecular Biology and Biotecnologies, and the Oncology Clinic of the Azerbaijan Medical University, Ministry of Health of the Republic of Azerbaijan. Informed written consent was obtained from all the participants (patients and volunteers) included in the study. Besides, ethical issues including plagiarism, data fabrication and double publication were completely avoided by the authors.

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