IRAQI Academic Scientific Journals
العراقية المجلات الاكاديمية العلمية
University of Kut Journal
ISSN (E): 2616 - 7808 II ISSN (P): 2414 - 7419
js.uokut.edu.iq
k.u.c.j.sci@alkutcollege.edu.iq
Vol. 11 , Issue 1 , June 2026

The Effect of Genetic Polymorphism of rs2283468 of IL-32 Gene in Patients with *Echinococcus granulosus*.

Riyadh Resan Saad [1] , Dr.Maysara samer khalaf [2] , Dr. Amal Hasan Atiyah [3]

Abstract

The E. granulosus is recognized as a biologically distinct subspecific variant or strain that may exhibit varying degrees of infectivity toward domestic animals and humans. This research examined the influence of the IL-32 gene polymorphism rs2283468 in patients with Echinococcus granulosus. This study has been ongoing from January 2024 until the end of May. Five ml of blood samples were collected from thirty subjects, including fifteen patients with cystic echinococcosis from the hospitals in Baghdad city and fifteen healthy persons as a control group, and fifteen hydatid cyst samples were collected from infected humans that had undergone a surgical operation. Conventional PCR with specific primers, followed by Sanger sequencing, was used to characterize the SNP. We analyzed the gene using Sanger sequencing for the SNP rs2283468 of IL-32, and found that patients with Echinococcus granulosus had genotype frequencies of AA (13.3%), CC (73.3%), and AC (13.4%). Also, in healthy people, there was AA (80.0%), CC (0.0%), and AC (20.0%). The CC genotype got the highest score in patients (73.3%) and the lowest score in healthy people (0.0%). This was in contrast to the dominant allele, which got the highest score in healthy people (80%) and the lowest score in patients (13.3%), with very different ($p<0.05$) and odd ratios (0.008). The C allele is more common in patients (75.0%) and least common in healthy people (16.67%), with a significant difference ($p< 0.05$).

Keywords: IL-32, Gene Polymorphism, rs2283468, Echinococcus granulosusm

Affiliation of Authors

[1, 2] College of Health and Medical Techniques, Middle Technical University, Iraq, Baghdad, 10047

[3] Institute of Medical Technology, Middle Technical University, Iraq, Baghdad, 10047

[1] riyadhresan@gmail.com
[2]dr.maysara-samer@mtu.edu.iq
[3] amhahy2017@gmail.com

[1] **Corresponding Author**

Paper Info.
Published: Jun. 2026

تأثير تعدد الأشكال الجينية rs2283468 لجين IL-32 في المرضى المصابين بطفيلي المشوكات الحبيبية

رياض ريسان سعد [1] ، د.ميسرة سمير خلف [2] ، د.امال حسن عطية [3]

المستخلص

تم التعرف على طفيلي المشوكة الحبيبية كنوع فرعي متميز بيولوجيًا قد يظهر درجات متفاوتة من القدرة على إصابة الحيوانات المنزلية والبشر.قامت هذه الدراسة بفحص تأثير تعدد أشكال الجين IL-32 rs2283468 لدى المرضى المصابين بداء الاكياس المائية وقد بدأت الدراسة في كانون الثاني 2024 واستمرت حتى نهاية ايار. تم جمع 5 مل من عينات الدم من ثلاثين نموذج ، شملوا خمسة عشر مريضًا مصابًا بداء الاكياس المائية من مستشفيات مدينة بغداد وخمسة عشر شخصًا أصحاء كمجموعة ضابطة، بالإضافة إلى جمع خمس عشرة عينة الاكياس المائية من المرضى الذين خضعوا لعملية جراحية. تم استخدام تقنية تضاعف البوليميراز المتسلسل التقليدي باستخدام بادئات محددة، تلتها عملية تسلسل سانجر لتوصيف تعدد الأشكال الجينية. قمنا بتحليل الجين باستخدام تسلسل سانجر لتعدد الأشكال الجينية rs2283468 من IL-32، ووجدنا أن المرضى المصابين بداء الاكياس المائية لديهم ترددات للأنماط الجينية AA (13.3%)، CC (73.3%)، و(13.4% AC). بينما لدى الأشخاص الأصحاء كان هناك AA (80.0%)، CC (0.0%)، و(20.0%) AC. حصل النمط الجيني CC على أعلى نسبة في المرضى (73.3%) وأدنى نسبة في الأصحاء (0.0%). في المقابل، حصل الأليل السائد على أعلى نسبة في الأصحاء (80%) وأدنى نسبة في المرضى (13.3%)، مع اختلاف كبير ($p<0.05$) ونسب الأرجحية (0.008). الأليل C أكثر شيوعًا بين المرضى (75.0%) وأقل شيوعًا بين الأصحاء (16.67%)، مع اختلاف معنوي ($p < 0.05$).الاستنتاجات: تشير الدراسة إلى أن جين IL-32، وبالأخص تعدد الأشكال rs2283468، مرتبط بتباين كبير في ترددات الأنماط الجينية بين المرضى المصابين بداء الاكياس المائية و الأصحاء.

الكلمات المفتاحية: انترلوكين 32، تعدد الأشكال الجينية، rs228346، طفيلي المشوكة الحبيبية

انتساب الباحثين

[1، 2] كلية التقنيات الصحية والطبية، جامعة التقنية الوسطى، العراق، بغداد، 10047

[3] معهد التقني الطبي، الجامعه التقنية الوسطى، العراق، بغداد، 10047

[1] riyadhresan@gmail.com
[2]dr.maysara-samer@mtu.edu.iq
[3] amhahy2017@gmail.com

[1] **المؤلف المراسل**

معلومات البحث
تأريخ النشر : حزيران 2026

Introduction

Cystic echinococcosis (CE) is a major parasitic zoonosis that affects humans and other animals [1].The larvae of *Echinococcus granulosus* cause the disease [2]. Epidemic echinococcosis infections not only endanger and damage the health of people and animals, but also significantly impede animal husbandry output and impact the growth of the national economy [3]. Adult worms lay their eggs in the small intestines of animals that eat meat, like dogs and wild cats. Larvae form inside the organs of intermediate hosts, usually plant-eating animals, after they eat the worm's eggs. Errors can affect people, leading to cystic echinococcosis (CE) [4].

E.granulosus is recognized as a biologically distinct subspecific variant or strain that may exhibit varying degrees of infectivity toward domestic animals and humans [5,6]. The World Health Organization (WHO) classifies human infections as "echinococcosis" and considers them to be among the neglected diseases, disorders, or conditions that are important for world health and for which control is advocated [7]. A category of parasitic diseases known as "echinococcosis" includes cystic (CE), alveolar (AE), and neotropical (NE) echinococcosis. It is believed that over a million people are infected with this disease at any given time[8, 9]. CE is the most common type of Echinococcus spp. infection worldwide, with significant rates of morbidity and relative mortality in human populations.[10] Morphological and biological investigations have yielded valuable data for strain identification, it is important to note that these characteristics might vary. Environmental and host factors may affect them, preventing them from representing distinctness at the genetic level [11]. Molecular methods offer the advantage of avoiding host or environmental variability, allowing for direct characterization of the parasite's genome [12]. On the chromosome or transcribed region of the polycistronic rRNA precursor transcript molecule, the internal transcribed spacer [ITS] is a DNA spacer located between the small subunit ribosomal RNA and large subunit ribosomal RNA genes. In eukaryotes, there are two ITS spacer DNAs: ITS1 is located between the 18S and 5.8S rRNA genes, and ITS2 is located between the 5.8S and 28S rRNA genes. Consequently, ITS1 and ITS2 are two spacer DNA sequences that are located between rRNA genes in eukaryotes [13] The first and second internal transcribed spacer sequences (ITS-1 and ITS-2) of rDNA function as precise genetic identifiers within the Echinococcus genus, according to prior study [14]. It is acknowledged that ITS2 is more conserved than ITS1 [15] because all ITS2 sequences share a similar secondary structure core, whereas ITS1 structures are extremely uncommon and only found in very small taxonomic groups. Regardless of the degree of conservation, structure-assisted comparison can yield higher resolution and robustness [16]. The IL-32 gene is located on the 16p13.3 region of the human chromosome. It has a length of about 1,200 base pairs and consists of eight exons [17] According to its alternative splicing sites, more than seven transcripts have been suggested. However, seven isoforms with nine exons were described to be translated from its messenger RNA transcript [18]. Lastly, IL-32θ was discovered from dendritic cells and Jurkat cells of human leukemia T cell line [19]. These isoforms exhibited distinct effects in different conditions. Among the seven IL-32 isoforms, IL-32γ is the most-studied isoform, which also has the longest amino acid sequence. IL-32 plays a vital modulator role in the pathogenesis of different

diseases. Its involvement has been reported in various cancers, infections, and autoimmune and inflammatory disorders [20] .Polymorphisms in IL-32 cytokines alter the activity of interleukins, which may also affect the function of cytokines, thereby dysregulating IL-32 expression [21].

Material and methods

This study has been ongoing from January 2024 until the end of May. Five ml of blood samples were collected from thirty subjects, including fifteen patients with cystic echinococcosis from the hospitals in Baghdad city and fifteen healthy persons as a control group, and hydatid cysts samples were collected from fifteen infected humans that underwent surgical operation. Conventional PCR with specific primers, followed by Sanger sequencing, was used to characterize the SNP.

Following the manufacturer's instructions for isolation, amplification, and sequencing, we extracted total cellular DNA from germinal layers and hydatid cyst fluid samples. Geneaid Taiwan was then used to purify the DNA, and blood samples were also treated with Geneaid. PCR was used in human and sample samples to identify the *E. granulosus* gene. This technique was implemented using this methodology. South Korea employed the PCR technique (ITS1-BD1 forward 4S reverse, ITS2-3S forward A28-reverse) genes were used to carry out this method that amplification by using the PCR technique, provided by South Korea as shown in Table (1).

Table (1): Primers (ITS1-BD1 forward 4S reverse, ITS2-3S forward A28-reverse) provided by South Korea

Primers	Sequence		Amplicon
ITS1-BD1 Forward 4S − reverse	F	5′GTCGTAACAAGGTTTCCGTA-3′	1000bp
	R	5′TCTAGATGCGTTCGAATGTCGATG-3′	
ITS2−3S-forward A28-reverse	F	5′GGTACCGGTGGATCACTCGGCTCG3′	1100bp
	R	5′GGTACCGGTGGATCACTCGGCTCG3′	

Sequencing of Study genes

The PCR results were sent to Macrogen Corporation, Korea, for Sanger sequencing utilizing the ABI3730XL automated DNA sequencer. The findings received were analyzed with Geneious software, concentrating on the SNP rs2283468 of IL-32.

Primers Used for Sequencing

Primers used for sequencing were instrumental in the DNA sequencing phase, particularly in the analysis of specific Single Nucleotide Polymorphisms (SNPs) such as rs2283468 in IL-32 as shown in Table (2).

Table (2): Primers used for sequencing SNPs rs2283468 in IL-32

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)
rs2283468-F	TGTAAAACGACGGCCAGTCCTCCAAATCTCGGGTTTAAG	60	1025
rs2283468-R	CAGGAAACAGCTATGACGCAAAGGTGGTGTCAGTATC		

Results and discussion

Results as shown in Table (3) and Figures (1) show the analysis of genes using Sanger sequencing for the SNPS: rs2283468 of IL-32.

Patients with *Echinococcus granulosus* have genotype frequencies of AA (13.3%), CC (73.3%), and AC (13.3%) for the SNP rs2283468 of the IL-32 gene. Additionally, in healthy individuals, the genotype frequencies were AA (80.0%), CC (0.0%), and AC (20.0%).

The CC genotype got the highest score in patients (73.3%) and the lowest score in healthy people (0.0%). This was in contrast to the dominant allele, which got the highest score in healthy people (80%) and the lowest score in patients (13.3%), with ratios that were also significantly different ($p<0.05$) and odd (0.008).

The C allele is the most common in patients (75.0%) and the least in healthy people (16.67%), with a non-significant difference ($p < 0.05$) as shown in Table (3).

Table (3): Comparative genotypes and allele frequency of SNP rs2283468 IL-32 gene between study groups by chi-square test and based on Hardy-Weinberg Equilibrium

Genotypes A= dominant allele C= recessive allele			**Groups**		**Total**	***P value***	**OR (C.I.)**
			Patients	**Healthy**			
rs2283468 IL-32 A>C	AA	N	2	12	14	**Reference value**	
		%	13.3%	80.0%	46.7%		
	CC	N	11	0	11	*P<0.05**	0.008 (0.0001-0.1410)
		%	73.3%	0.0%	36.7%		
	AC	N	2	3	5	*P>0.05*	0.25 (0.024-2.91)
		%	13.4%	20.0%	16.6%		
	Total	N	15	15	30	**OR= Odd Ratio C.I.= Confidence intervals**	
		%	100.0%	100.0%	100.0%		
	P value		*P<0.05**	*P<0.05**	*P<0.05**		
	Allele frequencies						

	A	N	4	15	19	*P<0.05**	0.066 (0.012-0.35)
		%	25.0%	83.33%	55.88%		
	C	N	12	3	15		
		%	75.0%	16.67%	44.12%		
	Total	N	16	18	34	**OR= Odd Ratio C.I.= Confidence intervals**	
		%	100.0%	100.0%	100.0%		
	P value		*P<0.05**	*P<0.05**	*P>0.05*		

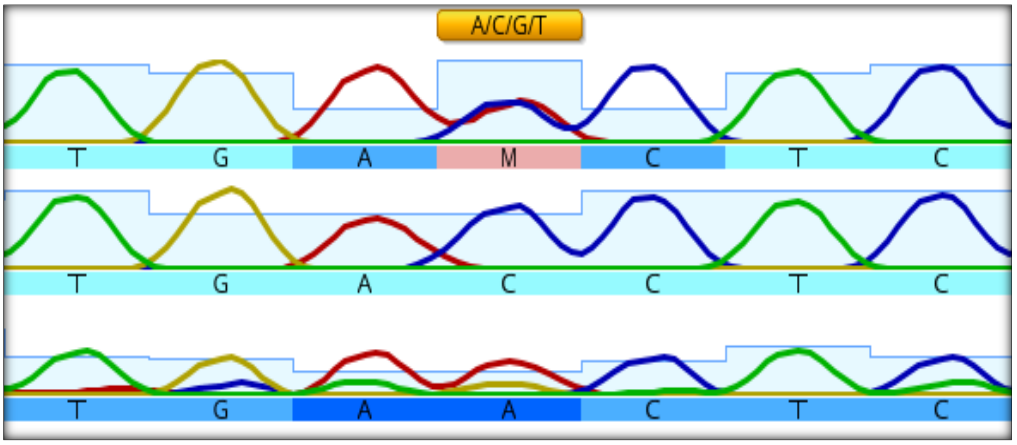

Figure (1): The study details the Sanger sequencing analysis of the rs2283468 single nucleotide polymorphism (SNP) in the IL32 gene. A single 'C' peak suggests the presence of a homozygous C allele, whereas a single 'A' peak indicates a homozygous A allele. The coexistence of both 'C' and 'A' peaks indicates a heterozygous C/A allele

The *E. granulosus genotypes* have the potential to infect humans. Therefore, different animals may be involved in the epidemiology of human echinococcurrence or hydatidosis, providing a suitable method to manage the infection depending on their transmission cycle in every area [22].

The human chromosome's 16p13.3 region is home to the IL-32 gene. It spans about 1,200 base pairs and comprises eight exons [17]. Polymorphisms in IL-32 cytokines can change how interleukins work and how cytokines work in general, which can throw off the production of IL-32 [21]. Table 1 demonstrates the variation in genotype and allele frequencies of the rs2283468 IL-32 polymorphism between patients with hydatid disease and healthy controls. The present results showed that the CC genotype and C allele scored a higher percentage in patients than the AA genotype and A allele. The results show that people with the CC genotype and C allele have low levels of IL-32. As a result, their immune systems are not as strong as those with the AA genotype and A allele.

The authors demonstrated a correlation between IL-32 and hosts' resistance to parasites. Variations in IL-32 may impact the host's vulnerability to Leishmania due to its genetic makeup. Researchers linked the IL32 rs4786370 genetic variant to a decreased risk of adult T-cell leukemia (ATL), while they linked IL32 rs4349147 to an increased vulnerability to localized cutaneous and mucosal leishmaniasis [23]. Previous research

indicates a correlation between bladder cancer susceptibility and prognosis in the Chinese Han population, specifically between Interleukin-32 gene polymorphisms rs12934561 and rs28372698 [24]. According to another study, individuals with the homozygous TT genotype and T allele of rs28372698 IL-32 have a higher risk of heart disease than those with the TT homozygosity and T allele [21]. the present study is considered the first study to determine the relationship between The Effect of Genetic Polymorphism of rs2283468 and CE patients because no references have been published about this subject. Therefore, further research is required to study the role of IL-32 gene Polymorphism in the pathogenesis of CE.

The ITS1 gene's banding patterns in human hydatid cysts on agarose gels as shown in Figures (2) and ITS2 gene's banding patterns in human hydatid cysts on agarose gels as shown in Figures (3).

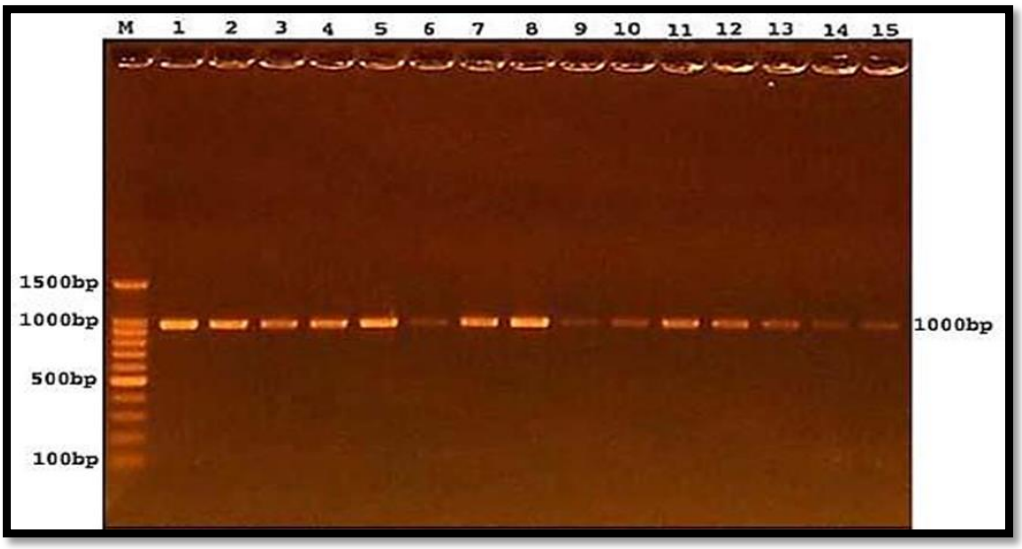

Figure (2): The ITS1 gene's banding patterns in human hydatid cysts on agarose gels, with molecular weight markers (100-bp) and numbered (2, 4, 5, 6, 7, 9, 11, 12, 13, 14, and 15) at the same molecular weight

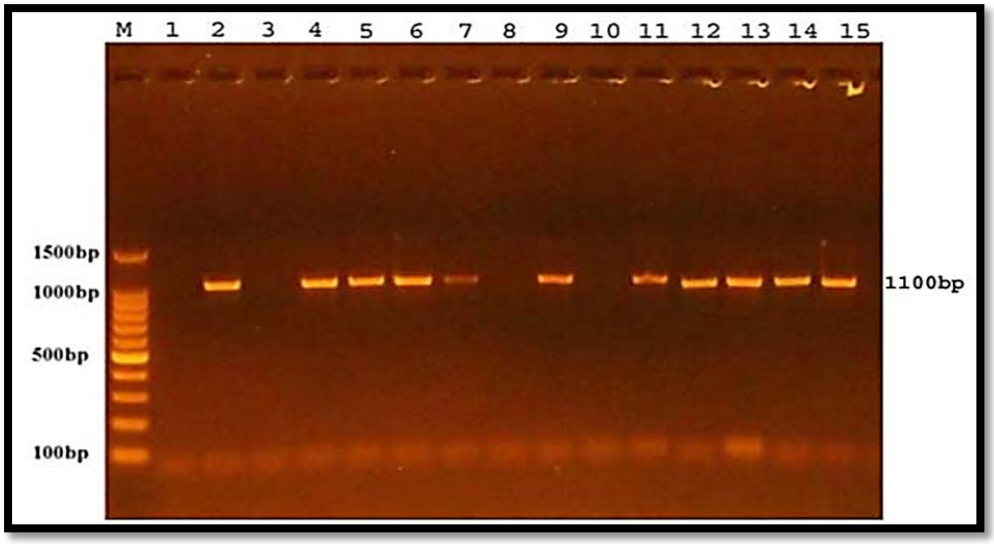

Figure (3): The ITS2 gene's banding patterns in human hydatid after electrophoresis on an agarose gel, with molecular size marker (100-bp), and numbered (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15) at molecular weight (1000-bp)

Conclusions

The study indicates that the IL-32 gene, specifically the rs2283468 polymorphism, is associated with significant variation in genotype frequencies between patients with cystic echinococcosis and healthy controls.

References

[1] Mandefro M, Tilahun B, Bayu Y, Zeryehun T. Prevalence of bovine hydatidosis and its economic importance in Adama Municipal Abattoir, Eastern Ethiopia. Ethiopian Veterinary Journal. 2019 Oct 7;23(1):24-41.

[2] Abdeta D, Desissa F, Deresa B, Moje N, Assefa Z. Epidemiology and Financial Loss of Bovine Hydatidosis in Cattle Slaughtered at Nekemte Municipal Abattoir, Ethiopia. Acta Para Global. 2014;5:133-8.

[3] Qian MB, Abela-Ridder B, Wu WP, Zhou XN. Combating echinococcosis in China: strengthening the research and development. Infectious Diseases of Poverty. 2017 Dec;6:1-5.

[4] Bonelli P, Dei Giudici S, Peruzzu A, Mura L, Santucciu C, Maestrale C, Masala G. Identification of Echinococcus granulosus genotypes G1 and G3 by SNPs genotyping assays. Pathogens. 2021 Jan 26;10(2):125.

[5] McManus DP, Smyth JD. Hydatidosis: changing concepts in epidemiology and speciation. Parasitology Today. 1986 Jun 1;2(6):163-8.

[6] Thompson RC. Biology and systematics of Echinococcus. Advances in parasitology. 2017 Jan 1;95:65-109.

[7] Casulli A. New global targets for NTDs in the WHO roadmap 2021–2030. PLoS Negl Trop Dis. 2021;15:e0009373

[8] Vuitton DA, McManus DP, Rogan MT, Romig T, Gottstein B, Naidich A, et al. World Association of Echinococcosis. International consensus on terminology to be used in the feld of echinococcoses. Parasite. 2020;27:41.

[9] World Health Organization. Echinococcosis - 17 may 2021. https://www. who.int/en/news-room/fact-sheets/detail/echinococcosis. Accessed 10 Dec 2021.

[10] Casulli A. Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. Lancet Glob Health. 2020;8:e470–1

[11] Hobbs RP, Lymbery AJ, Thompson RC. Rostellar hook morphology of Echinococcus granulosus (Batsch, 1786) from natural and experimental Australian hosts, and its implications for strain recognition. Parasitology. 1990 Oct;101(2):273-81.

[12] Bowles J, McManus DP. Molecular variation in Echinococcus. Acta Tropica. 1993 May 1;53(3-4):291-305.

[13] Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium, Fungal Barcoding Consortium Author List, Bolchacova E, Voigt K. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the national academy of Sciences. 2012 Apr 17;109(16):6241-6.

[14] Van Herwerden L., Gasser R.B., Blair D. 2000. ITS- 1 ribosomal DNA sequence variants are maintained in different species and strains of Echinococcus. International Journal for Parasitology 30: 157-169. doi:10.1016/s0020-7519(00)00002-3

[15] Schultz, J., Maisel, S., Gerlach, D., Müller, T., & Wolf, M. (2005). A common

core of secondary structure of the internal transcribed spacer 2 (ITS2) throughout the Eukaryota. *Rna*, *11*(4), 361-364.

[16] Koetschan, C., Kittelmann, S., Lu, J., Al-Halbouni, D., Jarvis, G. N., Müller, T., ... & Janssen, P. H. (2014). Internal transcribed spacer 1 secondary structure analysis reveals a common core throughout the anaerobic fungi (Neocallimastigomycota). PloS one, 9(3), e91928.

[17] Dahl CA, Schall RP, He HL, Cairns JS. Identification of a novel gene expressed in activated natural killer cells and T cells. Journal of immunology (Baltimore, Md.: 1950). 1992 Jan 15;148(2):597-603.

[18] Sohn DH, Nguyen TT, Kim S, Shim S, Lee S, Lee Y, et al. Structural Characteristics of Seven IL-32 Variants. *Immune Netw* (2019) 19(2):e8. doi: 10.4110/in.2019.19.e8.

[19] Kang JW, Park YS, Lee DH, Kim MS, Bak Y, Ham SY, et al. Interaction Network Mapping Among IL-32 Isoforms. *Biochimie* (2014) 101:248–51. doi: 10.1016/j.biochi.2014.01.013.

[20] Briukhovetska D, Dorr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in Cancer: From Biology to Therapy. *Nat Rev Cancer* (2021) 21(8):481–99. doi: 10.1038/s41568-021-00363-z

[21] Jin S, Liu X, Wang Y, Yu J, Jiang M. Effects of IL-32 polymorphisms and IL-32 levels on the susceptibility and severity of coronary artery disease. Journal of Clinical Laboratory Analysis. 2022 Jan;36(1):e24114.

[22] Guo, B., Zhao, L., Zhao, L., Mi, R., Zhang, X., Wang, B., ... & Zhang, Z. (2023). Survey and Molecular Characterization of Echinococcus granulosus sensu stricto from Livestock and Humans in the Altai Region of Xinjiang, China. Pathogens, 12(1), 134.

[23] Ye C, Zhang L, Tang L, Duan Y, Liu J, Zhou H. Host genetic backgrounds: the key to determining parasite-host adaptation. Frontiers in Cellular and Infection Microbiology. 2023 Aug 10;13:1228206.

[24] Yang J, Jian Z, Shen P, Bai Y, Tang Y, Wang J. Associations between Interleukin-32 gene polymorphisms rs12934561 and rs28372698 and susceptibilities to bladder cancer and the prognosis in chinese han population. Disease Markers. 2020(1):8860445.