Dmitrenko OP, et al., J Ophthalmic Clin Res 2022, 9: 097
DOI: 10.24966/OCR-8887/100097

HSOA Journal of Ophthalmology & Clinical Research

Research Article

Association of Polymorphism rs12778366 of the SIRT1 Gene with the Risk of Age-Related Macular Degeneration

Dmitrenko OP[1], Karpova NS[1]*, Abramova OI[2], Nurbekov MK[1] and Arshinova ES[1]

[1]Federal State Budgetary Institution "Research Institute of Pathology and Pathophysiology", 125315 Moscow, Russia

[2]Federal State Budgetary educational institution of continuing professional education "Russian Medical Academy of Continuing Professional Education" of the Ministry of Health of the Russian Federation, 125993 Moscow, Russia

Abstract

Purpose: Age-Related Macular Degeneration (AMD) is a complex multifactorial disease, which consists of various genetic, environmental and constitutional factors and is characterized by damage to the macular zone of the retina. The AMD is one of the most common causes of blindness and poor vision in people of a senior age group. Aging is regarded as one of the most significant factors predisposing to age-related macular generation. Sirtuins, in particular SIRT1, are a family of signaling proteins that play an important role in the aging process. The SIRT1 is the most studied protein in the topic of aging and its level of expression plays an important role in the AMD development. For these reasons, we evaluated the relationship of the rs12778366 polymorphism with the risk of AMD.

Methods: The study used genomic DNA isolated and purified from buccal epithelial cells from 384 people (192 AMD patients and 192 non-AMD patients). Genotyping of the selected polymorphisms was conducted by real-time PCR using the TaqMan competing probe technology.

Results: The C allele in the additive inheritance model and the TC heterozygous genotype in the codominant and recessive models serve as the genetic factor predisposing to this disease (p<0,001, OR: 2,121, 95% of CI: 1.435-3.133; p<0.001, OR: 2.499, 95% of CI: 1.595-3.915; p<0.001, OR: 2.507, 95% of CI: 1.612-3.900). In addition, the C allele was more common in women with AMD and in AMD patients over 65 years of age, which may be associated with a higher risk of AMD.

Conclusion: Our study discovered a significant association between rs12778366 polymorph locus in SIRT1 gene with AMD.

Keywords: Age-related macular degeneration; Gene polymorphism; SIRT1

***Corresponding author:** Karpova NS, Federal State Budgetary Institution "Research Institute of Pathology and Pathophysiology", Moscow, Russia, E-mail: nataliiakarpova.sp@gmail.com

Citation: Dmitrenko OP, Karpova NS, Abramova OI, Nurbekov MK, Arshinova ES (2022) Association of Polymorphism rs12778366 of the SIRT1 Gene with the Risk of Age-Related Macular Degeneration. J Ophthalmic Clin Res 9: 097.

Received: August 08, 2022; **Accepted:** August 19, 2022; **Published:** August 23, 2022

Introduction

Age-Related Macular Degeneration (AMD) is a disease that is characterized by the retinal macular zone impairment, which is the most common cause of blindness among the elderly in medium and high income countries [1,2]. The prevalence of AMD increases with age of over 65 years. Women over the age of 75 have 2 times higher risk of obtaining the disease than men [3]. The disease incidence ranges from 2% to 20%. This number is expected to grow as the world population ages and to reach 288 million by 2040 [4]. In the Russian Federation, the disease prevalence is 15 people per 1000 population and every twentieth inhabitant is at risk for AMD [5]. Currently, the disease onset becomes reported at a much earlier time and is now diagnosed among middle-aged people, which results in a growing disability among the working population [1].

Aging is an inevitable physiological process and is regarded as one of the most significant factors predisposing to age-related macular generation because this disease incidence increases among individuals older than 60. It was found by the empirical research that sirtuins play an important role in the ocular aging and influence AMD pathogenesis development [6-8].

Sirtuins (SIRT1-SIRT7) belong to the family of Class III histondeacylases, their catalytic activity depends on NAD+ level and NAD+/NADH changes over time [9].The SIRT1is most studied member of (SIRT1-SIRT7) family in the context of aging. Its expression is known to decrease as a person becomes older, hence the change in SIRT1 level may be the key intermediate link in the development mechanism of a number of age-associated diseases [10-12]. SIRT1 catalyzes deacylation of various nuclear and cytoplasm proteins by interacting with the transcription factors, such as PGC-1a, NFKB, p53 and FoxO, which is crucial for the life functioning at the cellular level and for controlling such biological processes as inflammation, metabolism, redox homeostasis, DNA repair, cell proliferation and aging [13,14]. SIRT1 is expressed in the ocular retina, including ganglion cells, photoreceptors and RPE cells, and its retinal expression is variable [15,16]. Numerous studies suggest that impaired regulation of SIRT1 activity is associated with retinal aging and such ocular diseases as cataract, age-related macular degeneration, diabetic retinopathy and glaucoma [6,7,14].

The function of the SIRT1 protein is affected by the expression of the SIRT1 gene, which is located on the 10th chromosome in the q21.3 region and contains 11 exons. Previous studies have shown that point

mutations in promoters are more common than in coding regions of a gene and can affect its expression [17].

The rs12778366 polymorphism in the SIRT1 gene promoter region, according to few studies, is associated with oncological, cardiovascular and neurodegenerative diseases, type 2 diabetes mellitus, macular degeneration and life expectancy [18-20].

By reviewing related scientific literature, we hypothesize that carriers of different genotypes in polymorphic regulatory gene sites have a different baseline level of SIRT1 gene expression and that SIRT1 gene rs12778366 polymorphism may serve as an important AMD predictor.

Materials and Methods

Research objects

The design of the study and the usage of human material were approved by the Local Ethics Committee of the Research Institute of General Pathology and Pathophysiology. The study was conducted in accordance with the recommendations on strengthening research reporting by the Genetic Association (STREGA), an extension of the STROBE statement [21]. The study included patients who underwent a standard ophthalmological examination in the ophthalmological department of the State Medical University named after S.P. Botkin DZM G. Moscow in 2019-2021. All respondents were Russian native speakers of undetermined ethnicity (in accordance with the ethical standards of the local medical register) and gave signed informed consent forms in accordance with the Helsinki Declaration. The diagnosis of AMD was established in accordance with the recommendations of the American Academy of Ophthalmology (AAO) and based on the criteria of the Russian national clinical guidelines "Age-related macular degeneration: [1,2]. The exclusion criteria were: acute and chronic diseases in the exacerbation stage of the visual organs, glaucoma, uveitis of various etiologies, complete complicated cataract, retinal detachment, iris rubeosis. The study did not include patients with autoimmune and oncological processes of any localization. The study did not include women with multiple pregnancies, other pregnancy complications, as well as disorders affecting glucose metabolism. QUANTO quantification software (Version 1.2.4, https://bio.tools/QUANTO), which takes into account the frequency of SNPs in the population and the prevalence of the disease [22]. In accordance with the above parameters, a sample size of 136 case-control pairs is required to identify the association between the selected polymorphisms and the risk of AMD.

The study included 192 patients with AMD older than 45 years. The control group consisted of 192 people who had no ophthalmological pathology during the examination corresponding age. There were no significant differences in gender and age between patients with AMD and controls ($p > 0.05$) (Table 1).

Characteristic	Group		*P* value
	AMD, n = 192	Control, n = 192	
Men, n (%)	50 (26.04)	46 (23.9)	0.4468*
Women, n(%)	142 (73.96)	146 (76.1)	
Age, M ± S, years	70.96 ± 9.72	69.23 ± 11.91	0.4596*

Table 1: Demographic characteristics of the study population.

*not significant: $p > 0.05$

For analysis, the study population was divided into groups by gender and according to their age: younger than 65 years; and 65 years and older.

Genomic DNA extraction and polymerase chain reaction (PCR)

The DNA was extracted from buccal epithelial cells and purified with a genomic DNA extraction kit (Evrogen LLC, Russia) according to the kit specification. The high-molecular DNA was stored at -20ºC.

The quantity and quality of the isolated DNA was assessed using NanoDrop 1000 spectrophotometer in accordance with accepted standards.

Genotyping was performed in real-time using the technology of competing TaqMan probes according to the method taken from the literature. Primers and Taq-man probes were synthetically produced by Evrogen LLC, Russia (Table 2).

SNP	Oligonucleotidetypeandsequences
rs12778366	Forward 1: *CCCCACGCAACCAAAGAT* Revers: *ATCGCTAAGGTCCTATCTACA* Taq-Man Probe for T allele: *FAM-CTGGTCACCACTATTCATTTCT-GA-BHQ1* Taq-Man Probe for C allele: *HEX-TGGTCACCACTGTTCATTCT-GAA-BHQ1*

Table 2: Sequences of oligonucleotides for RT-PCR of the rs12778366.

The reaction mixture for RT-PCR for one 25 µL sample contained 20 ng DNA, 5 µL x5 qPCRmix-HS (Evrogen LLC, Russia), 200 µM forward primer, 200 µM reverse primer, 100 µM each of Taq-man probes.

Amplification was carried out in the CFX 96 programmable amplifier (Bio-Rad, U.S.A.) with the subsequent thermocycling parameters for rs12778366: initial denaturation for 5 minutes at 95°C; then 40 cycles including denaturation at 95°C for 30 seconds, at 60°C for 30 seconds, at 72°C for 30 seconds with subsequent fluorescence pickup. The obtained data was examined using the CFX Manager TM software (Bio-Rad).

To eliminate genotyping errors, 30% of randomly selected samples were re-genotyped and the results obtained were additionally evaluated.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, IL, USA). Continuous data were shown as mean ± standard deviation (±SD) if normally distributed. The difference in age between groups was analyzed using Student's t-test. The Hardy–Weinberg equilibrium test was performed using the chi-square test in cases and controls separately for each variant before association analysis. The variation in allele and genotype frequencies between groups was analyzed using Pearson's chi-square test or the Fisher exact test. Logistic regression analysis was used to evaluate associations between SNP genotypes and alleles and GDM risk by calculating odds ratios (ORs) and their 95% confidence intervals (CIs). The anticipated risk factor was regarded as significant for pathology if OR adjusted by CI was greater than 1. The level of significance was considered significant at $p < 0.05$.

Results

The distribution of the analyzed frequencies of genotypes and alleles rs12778366 of the SIRT1 gene in AMD patients and the control group corresponded to the Hardy-Weinberg equilibrium. The evaluation of the polymorphism rs12778366 of the SIRT1 gene revealed that the distribution of genotypes varies between AMD and control groups (57.29%, 40.63% and 2.08% vs. 77.08%, 21.88% and 1.04%, p = 0.0002) (Table 3).

Genotypesandalleles	AMD group, n (%)	*P* value HWE	Control group, n (%)	*P* value HWE	*P* value
Genotypes					
TT	110 (57.29)		148 (77.08)		
TC	78 (40.63)		42 (21.88)		
CC	4 (2.08)		2 (1.04)		
Total	192 (100)	0,288	192 (100)	0.267	χ^2 =17.0636 p = 0.0002
Alleles					
T	298 (77.61)		338 (88,02)		
C	86 (22.39)		46 (11,98)		

Table 3: Distribution of genotypes and alleles of polymorphisms rs12778366 of the SIRT1 gene in the group of patients with AMD and the control group.

Bold values show statistical significance.

Comparison of the frequency of SIRT1 rs12778366 genotypes in men between patients with AMD and the control group revealed differences without confirmation of statistical significance. Statistically significant differences in the distribution of genotypes were revealed between women with AMD and women of the control group (56.34%, 41.55% and 2.11% vs. 79.45%, 19.84% and 0.71%, p< 0.001). The C allele was more common in women with AMD (23.23% vs. 10.62%, p < 0.001), which may be associated with a higher risk of AMD in women than in men (Table 4).

Genotype/ Allele	Men		*P* value	Women		*P* value
	AMD group, n=50, n (%)	Control group, n=46, n (%)		AMD group, n=142, n (%)	Control group, n=146, n (%)	
TT	30 (60.00)	32 (69.57)	0.599	80 (56.34)	116 (79.45)	**<0.001**
TC	19 (38.00)	13 (28.26)		58 (41.55)	29 (19.84)	
CC	1 (2.00)	1 (2.17)		3 (2.11)	1 (0.71)	
T	79 (79.00)	77 (83.69)	0.405	218 (76.76)	261 (89.38)	**<0,001**
C	21 (21.00)	15 (16.31)		64 (23.23)	31 (10.62)	

Table 4: Frequency of SIRT1 rs12778366 genotypes and alleles in patients with AMD and control group by gender.

Bold values show statistical significance.

Comparison of the frequency of SIRT1 rs12778366 genotypes between patients with AMD younger than 65 years and the control group revealed differences without confirmation of statistical significance (p > 0.05). Statistically significant differences in the distribution of genotypes were revealed between patients with AMD older than 65 years and subjects of the control group of the same age (59.42%, 39.13% and 1.45% vs. 87.5%, 11.61% and 0.89%, p < 0.001). The C allele was more common in AMD patients over 65 years of age (21.02% vs. 6.7%, p < 0.001) (Table 5).

While analyzing associations using logistic regression analysis, it was found that the heterozygous TC genotype is associated with a 2,5-fold increase in the likelihood of developing AMD in the codominant and recessive genetic models (p < 0.001) (Table 6). Allele C increased the probability of developing AMD by 2,1 times according to the additive model (OR = 2.121; CI: 1.435-3.133; p < 0.001).

Genotype/ Allele	<65 years		*P* value	≥65 years		*P* value
	AMD group (n=54), n (%)	Control group (n=80), n (%)		AMD group (n=138), n (%)	Control group (n=112), n (%)	
TT	28 (51.85)	50 (62.50)	0.174	82 (59.42)	98 (87.50)	**<0.001**
TC	24 (44.45)	29 (36.25)		54 (39.13)	13 (11.61)	
CC	2 (3.70)	1 (1.25)		2 (1.45)	1 (0.89)	
T	80 (78.43)	129 (71.67)	0.204	218 (78.98)	209 (93.30)	**<0.001**
C	28 (21.57)	31 (28.33)		58 (21.02)	15 (6.70)	

Table 5: Frequency of SIRT1 rs12778366 genotypes and alleles in patients with AMD and the control patients by age.

Bold values show statistical significance.

Model ofinheritance	Genotypes	AMD group, n=192	Control group, n=192	OR (95% of CI)	chi2	P
Codominant	TT	110	148	**0.929** 0.163-5.282	0.01	0.933
	TC	78	42	**2.499** 1.595-3.915	16.39	**<0.001**
	CC	4	2	**2.691** 0.484-14.956	1.38	0.240
Dominant	TT + TC/ CC	188/4	190/2	**0.495** 0.090-2.733	0.68	0.410
Recessive	TT/TC + CC	110/82	148/44	**2.507** 1.612-3.900	17.06	**<0.001**
Additive	T	298	338	**0.472** 0.319-0.697	14.64	**<0.001**
	C	86	46	**2.121** 1.435-3.133	14.64	**<0.001**

Table 6: Association of genotypes of polymorphisms rs12778366 of the SIRT1 gene with AMD.

Bold values show statistical significance.

Discussion

In the current study, we analyzed the association between rs12778366 of the SIRT1 gene and the risk of AMD. We found that rs12778366 of the SIRT1 gene was associated with the risk of AMD in our population. It was found that the heterozygous TC genotype is associated with a 2,5-fold increase in likelihood of developing AMD in the codominant and recessive genetic models. The frequency of the C allele rs12778366 was significantly higher in patients with AMD compared to the control group. In addition, the C allele was more commonly found in women with AMD and in AMD patients over 65 years of age. Therefore, we can suggest that the C allele can be associated with a higher risk of AMD.

Age-related macular degeneration is a common multigenic disease with autosomal dominant inheritance type, in which genetic, environmental and lifestyle factors contribute to the disease risk. While genetic factors have the significant influence of the former accounting for up to 71% of the disease variability [23].

According to the International AMD Genomics Consortium (IAMDGC), 52 genetic versions are associated with the risk of AMD late stage and localized in 34 loci, 16 of which were not regarded as associated with AMD, have been identified so far [24]. Nowadays it is considered that the pathogenesis of AMD is based on dysfunction of RPE cells, which is caused by impaired metabolism in mitochondria, oxidative stress, inflammation, changes in the extracellular matrix, impaired lipid metabolism and angiogenesis in the retinal capillary network [25-27]. Previous studies have shown the regulatory role of SIRT1 in these pathological processes [28-32].

The rs12778366 of SIRT1 gene association with AMD is poorly studied. At the moment, there are two research papers, which focus on the relationship of this polymorphism with the disease risk. The study by Chen et al., demonstrated that rs12778366 was associated with AMD in the SIRT1 promoter region in homozygous carriers of the C minor allele in the Chinese population of Hang (p=0.036) [18]. Liutkeviciene et al., discovered that there is a close to statistically significant association with AMD in the Lithuanian population by the C allele of SIRT1 gene rs12778366 polymorphism [33]. The association between SIRT1 gene polymorph rs12778366 locus and AMD in the Russian population has never been studied before.

Conclusion

Our study discovered a significant association between rs12778366 polymorph locus in SIRT1 gene with the disease in the codominant, recessive and the additive models for AMD. The main limitation of this study was the small sample size. However, our data indicates the need for further research of the association between rs12778366 polymorphism in SIRT1 gene and AMD, with a simultaneous study of other SIRT1 genetic variations that may also contribute to this disease onset. It will provide better assessment of the contribution of rs12778366 polymorphism and other polymorphic loci of SIRT1 gene into AMD origination.

References

1. Moscow (2021) Age-related macular degeneration. Russian clinical guidelines. Moscow, Russia.
2. Flaxel CJ, Adelman RA, Bailey ST, Fawzi A, Lim JI, et al. (2020) Age-Related Macular Degeneration Preferred Practice Pattern® [published correction appears in Ophthalmology. Ophthalmology 127: 1-65.
3. Holz F, Pauleikhoff D, Spaide RF, Bird AC (2004) Age-related macular degeneration. Springer-Verlag, Berlin, Heidelberg, Germany.
4. Wong WL, Su X, Li X, Cheung CM, Klein R, et al. (2014) Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health 2: 106-116.
5. Avetisov SE, Egorov EA, Moshetov LK (2019) Ophthalmology. National Leadership, Russia.
6. Mimura T, Kaji Y, Noma H, Funatsu H, Okamoto S (2013) The role of SIRT1 in ocular aging. Exp Eye Res 116: 17-26.
7. Zhou M, Luo J, Zhang H (2018) Role of Sirtuin 1 in the pathogenesis of ocular disease (Review). Int J Mol Med 42: 13-20.
8. Wątroba M, Szukiewicz D (2016)The role of sirtuins in aging and age-related diseases. Adv Med Sci 61: 52-62.
9. Jadeja RN, Thounaojam MC, Bartoli M, Martin PM (2020) Implications of NAD$^+$ Metabolism in the Aging Retina and Retinal Degeneration. Oxid Med Cell Longev 2020: 2692794.
10. Zhao L, Cao J, Hu K, He X, Yun D, et al. (2020) Sirtuins and their Biological Relevance in Aging and Age-Related Diseases. Aging Dis 11: 927-945.
11. Yu M, Zhang H, Wang B, Zhang Y, Zheng X, et al. (2021) Key Signaling Pathways in Aging and Potential Interventions for Healthy Aging. Cells 10: 660.
12. Blasiak J, Pawlowska E, SobczukA, Szczepanska J, Kaarniranta K (2020) The Aging Stress Response and Its Implication for AMD Pathogenesis. Int J MolSci 21: 8840.
13. Carafa V, Rotili D, Forgione M, Cuomo F, Serretiello E, et al. (2016) Sirtuin functions and modulation: from chemistry to the clinic. Clin Epigenetics 8: 61.
14. Balaiya S, Amero KKA, Kondkar AA, Chalam KV (2017) Sirtuins expression and their role in retinal diseases. Oxid Med Cell Longev 2017: 3187594.
15. Jaliffa C, Ameqrane I, Dansault A, Leemput J, Vieira V, et al. (2009) Sirt1 involvement in rd10 mouse retinal degeneration. Investigative Ophthalmology & Visual Science 50: 3562-3572.
16. Bhattacharya S, Chaum E, Johnson DA, Johnson LR (2012) Age-related susceptibility to apoptosis in human retinal pigment epithelial cells is triggered by disruption of p53-Mdm2 association. Invest Ophthalmol Vis Sci 53: 8350-8366.
17. Deihimi T, Niazi A, Ebrahimi M, Kajbaf K, Fanaee S, et al. (2012) Finding the undiscovered roles of genes: an approach using mutual ranking of coexpressed genes and promoter architecture-case study: dual roles of thaumatin like proteins in biotic and abiotic stresses. Springerplus 1: 30.
18. Chen C, Zhou M, Ge Y, Wang X (2020) SIRT1 and aging related signaling pathways. Mech Ageing Dev 187: 111215.
19. Chen Z, Zhai Y, Zhang W, Teng Y, Yao K (2015)SingleNucleotide Polymorphisms of the Sirtuin 1 (SIRT1) Gene are Associated Withage-Related Macular Degeneration in Chinese Han Individuals: A Case-ControlPilot Study. Medicine (Baltimore) 94: 2238.
20. Figarska SM, Vonk JM, Boezen HM (2013) SIRT1 polymorphism, long-term survival and glucose tolerance in the general population. PLoS ONE 8: 58636.
21. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, et al. (2009) STrengthening the REporting of Genetic Association Studies (STREGA)--an extension of the STROBE statement. Genet Epidemiol 33: 581-598.
22. Gauderman WJ (2002) Sample size requirements forassociation studies of gene-gene interaction. Am J Epidemiol 155: 478-484.
23. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC (2005) The US twin study of age-related macular degeneration: relative roles ofgenetic and environmental influences. Arch Ophthalmol 123: 321-327.
24. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S et al. (2016) A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet 48: 134-143.
25. Ao J, Wood JP, Chidlow G, Gillies MC, Casson RJ (2018) Retinal pigment epithelium in the pathogenesis of age-related macular degeneration and photobiomodulation as a potential therapy? Clin Exp Ophthalmol 46: 670-686.
26. Wang S, Wang X, Cheng Y, Ouyang W, Sang X, et al. (2019) Autophagy Dysfunction, Cellular Senescence, and Abnormal Immune-Inflammatory Responses in AMD: From Mechanisms to Therapeutic Potential. Oxid Med Cell Longev 2019: 3632169.
27. Ferrington DA, Fisher CR, Kowluru RA (2020) Mitochondrial Defects Drive Degenerative Retinal Diseases. Trends Mol Med 26: 105-118.
28. Balaiya S, Khetpal V, Chalam KV (2012) Hypoxiainitiates sirtuin1-mediated vascular endothelial growth factor activation inchoroidal endothelial cells through hypoxia inducible factor-2α. Mol Vis 18: 114-120.

29. Golestaneh N, Chu Y, Cheng SK, Cao H, Poliakov E, et al. (2016) Repressed SIRT1/PGC-1α pathway and mitochondrial disintegration in iPSC-derived RPE disease model of age-related macular degeneration. J Transl Med 14: 344.

30. Zhang M, Jiang N, Chu Y, Postnikova O, Varghese R, et al. (2020) Dysregulated metabolic pathways in age-related macular degeneration. Sci Rep 10: 2464.

31. Cao L, Liu C, Wang F, Wang H (2013) SIRT1 negatively regulates amyloid-beta-induced inflammation via the NF-κB pathway. Braz J Med Biol Res 46: 659-69.

32. Li L, Wei W, Zhang Y, Tu G, Zhang Y, et al. (2015) SirT1 and STAT3 protect retinal pigmented epithelium cells against oxidative stress. Mol Med Rep 12: 2231-2238.

33. Liutkeviciene R, Vilkeviciute A, Kriauciuniene L, Deltuva VP (2019) SIRT1 rs12778366, FGFR2 rs2981582, STAT3 rs744166, LIPC rs10468017, rs493258 and LPL rs12678919 genotypes and haplotype evaluation in patients with age-related macular degeneration. Gene 686: 8-15.

Advances In Industrial Biotechnology | ISSN: 2639-5665

Advances In Microbiology Research | ISSN: 2689-694X

Archives Of Surgery And Surgical Education | ISSN: 2689-3126

Archives Of Urology

Archives Of Zoological Studies | ISSN: 2640-7779

Current Trends Medical And Biological Engineering

International Journal Of Case Reports And Therapeutic Studies | ISSN: 2689-310X

Journal Of Addiction & Addictive Disorders | ISSN: 2578-7276

Journal Of Agronomy & Agricultural Science | ISSN: 2689-8292

Journal Of AIDS Clinical Research & STDs | ISSN: 2572-7370

Journal Of Alcoholism Drug Abuse & Substance Dependence | ISSN: 2572-9594

Journal Of Allergy Disorders & Therapy | ISSN: 2470-749X

Journal Of Alternative Complementary & Integrative Medicine | ISSN: 2470-7562

Journal Of Alzheimers & Neurodegenerative Diseases | ISSN: 2572-9608

Journal Of Anesthesia & Clinical Care | ISSN: 2378-8879

Journal Of Angiology & Vascular Surgery | ISSN: 2572-7397

Journal Of Animal Research & Veterinary Science | ISSN: 2639-3751

Journal Of Aquaculture & Fisheries | ISSN: 2576-5523

Journal Of Atmospheric & Earth Sciences | ISSN: 2689-8780

Journal Of Biotech Research & Biochemistry

Journal Of Brain & Neuroscience Research

Journal Of Cancer Biology & Treatment | ISSN: 2470-7546

Journal Of Cardiology Study & Research | ISSN: 2640-768X

Journal Of Cell Biology & Cell Metabolism | ISSN: 2381-1943

Journal Of Clinical Dermatology & Therapy | ISSN: 2378-8771

Journal Of Clinical Immunology & Immunotherapy | ISSN: 2378-8844

Journal Of Clinical Studies & Medical Case Reports | ISSN: 2378-8801

Journal Of Community Medicine & Public Health Care | ISSN: 2381-1978

Journal Of Cytology & Tissue Biology | ISSN: 2378-9107

Journal Of Dairy Research & Technology | ISSN: 2688-9315

Journal Of Dentistry Oral Health & Cosmesis | ISSN: 2473-6783

Journal Of Diabetes & Metabolic Disorders | ISSN: 2381-201X

Journal Of Emergency Medicine Trauma & Surgical Care | ISSN: 2378-8798

Journal Of Environmental Science Current Research | ISSN: 2643-5020

Journal Of Food Science & Nutrition | ISSN: 2470-1076

Journal Of Forensic Legal & Investigative Sciences | ISSN: 2473-733X

Journal Of Gastroenterology & Hepatology Research | ISSN: 2574-2566

Journal Of Genetics & Genomic Sciences | ISSN: 2574-2485

Journal Of Gerontology & Geriatric Medicine | ISSN: 2381-8662

Journal Of Hematology Blood Transfusion & Disorders | ISSN: 2572-2999

Journal Of Hospice & Palliative Medical Care

Journal Of Human Endocrinology | ISSN: 2572-9640

Journal Of Infectious & Non Infectious Diseases | ISSN: 2381-8654

Journal Of Internal Medicine & Primary Healthcare | ISSN: 2574-2493

Journal Of Light & Laser Current Trends

Journal Of Medicine Study & Research | ISSN: 2639-5657

Journal Of Modern Chemical Sciences

Journal Of Nanotechnology Nanomedicine & Nanobiotechnology | ISSN: 2381-2044

Journal Of Neonatology & Clinical Pediatrics | ISSN: 2378-878X

Journal Of Nephrology & Renal Therapy | ISSN: 2473-7313

Journal Of Non Invasive Vascular Investigation | ISSN: 2572-7400

Journal Of Nuclear Medicine Radiology & Radiation Therapy | ISSN: 2572-7419

Journal Of Obesity & Weight Loss | ISSN: 2473-7372

Journal Of Ophthalmology & Clinical Research | ISSN: 2378-8887

Journal Of Orthopedic Research & Physiotherapy | ISSN: 2381-2052

Journal Of Otolaryngology Head & Neck Surgery | ISSN: 2573-010X

Journal Of Pathology Clinical & Medical Research

Journal Of Pharmacology Pharmaceutics & Pharmacovigilance | ISSN: 2639-5649

Journal Of Physical Medicine Rehabilitation & Disabilities | ISSN: 2381-8670

Journal Of Plant Science Current Research | ISSN: 2639-3743

Journal Of Practical & Professional Nursing | ISSN: 2639-5681

Journal Of Protein Research & Bioinformatics

Journal Of Psychiatry Depression & Anxiety | ISSN: 2573-0150

Journal Of Pulmonary Medicine & Respiratory Research | ISSN: 2573-0177

Journal Of Reproductive Medicine Gynaecology & Obstetrics | ISSN: 2574-2574

Journal Of Stem Cells Research Development & Therapy | ISSN: 2381-2060

Journal Of Surgery Current Trends & Innovations | ISSN: 2578-7284

Journal Of Toxicology Current Research | ISSN: 2639-3735

Journal Of Translational Science And Research

Journal Of Vaccines Research & Vaccination | ISSN: 2573-0193

Journal Of Virology & Antivirals

Sports Medicine And Injury Care Journal | ISSN: 2689-8829

Trends In Anatomy & Physiology | ISSN: 2640-7752