How to Cite:
Alnaji, H. A., Hassan, A. H., & Omran, R. (2022). Genetic polymorphism of AKR1B1 with diabetic retinopathy: A pilot study. *International Journal of Health Sciences*, *6*(S3), 8310–8315. https://doi.org/10.53730/ijhs.v6nS3.7898

Genetic polymorphism of AKR1B1 with diabetic retinopathy: A pilot study

Haider Ali Alnaji
Medical Laboratory Technology Department, College of Medical Technology, The Islamic University, Najaf, Iraq | Biology Department, College of Science, Babylon University, Babylon, Iraq
Email: Hgeoda995@gmail.com

Azhar Hamza Hassan
Biology Department, College of Science, Babylon University, Babylon, Iraq
Email: Sci.azhar.hamza@uobabylon.edu.iq

Rabab Omran
Biology Department, College of Science, Babylon University, Babylon, Iraq
Email: omranaljelawi@gmail.com

***Abstract*---** Background. Diabetic retinopathy (DR) is one of the most common consequences of diabetes. Retinopathy risk may be affected by genetic polymorphisms in the AKR1B1 gene. Method. One hundred thirty-four people with diabetes enrolled in the current study with 36 health control. An ophthalmological evaluation was done along with genotyping for the AKR1B1 rs9640883 SNP for all participants. Results. The result of genotyping the rs9640883 SNP in AKR1B1 with diabetic retinopathy is a GG pattern in all recruited cases with DR or cases without this complication (134 patients) and 36 controls. The mutant AA genotype and the heterozygote GA genotype were absent in the samples. Conclusion. rs9640883 polymorphism was absent in our samples of DR, and it suggests the presence of the wild type in this locus. The A allele of rs9640883 polymorphism was absent in our samples of DR, and it suggests the presence of the wild type (G allele) in this locus.

***Keywords*---**diabetic retinopathy, AKR1B1, rs9640883, polymorphism.

Introduction

Diabetic retinopathy (DR) is one of the most common consequences of diabetes(Ting et al., 2016) Many patients with diabetes develop serious problems

such as retinal detachment, neovascular glaucoma, or diabetic macular edema. DR is the leading cause of vision loss in the working-age population worldwide. Progression of DR is thought to be genetically influenced, particularly the development from non-proliferative to more proliferative DR type (Pinazo-Durán et al., 2016; Romero-Aroca et al., 2016). However, not every patient with diabetes will develop microvascular problems(Wang et al., 2003). It is still unknown and poorly understood what factors led to the development of this complicated disease. Evidence suggests both environmental and genetic factors contribute to emerging DR(Priščáková et al., 2016). Studies on genetic factors use the comparing approach in which the frequency of a particular genetic variant in subjects with or without DR. one of the candidate genes for DR. is AKR1B1 encoded aldose reductase (ALR2); encoded aldose reductase is an essential enzyme in the polyol pathway (converts glucose to sorbitol) (Brownlee, 2005). Sorbitol accumulation leads to the destruction of the retina; by beginning the oxidative stress and ending in the production of microaneurysms, thickening the basement membrane of the retina(Priščáková et al., 2016). Three distinct DR-associated ALR2 polymorphisms have been found in various populations(Abhary et al., 2010; Li et al., 2019; S. Mogilevskyy et al., n.d.; S. I. Mogilevskyy & Bushuieva, 2017). However, conflicting data have been found in different publications. Studies show a higher risk of diabetic retinopathy related to a certain allele of polymorphisms, whereas the converse was shown in other studies (Cao et al., 2018; Demaine, 2003; Kao et al., 2000). In our present study, we aimed to identify the presence of genetic variant rs9640883 in our individual and the association or significance concerning diabetic retinopathy.

Materials and Methods

Subject

One hundred thirty-four people with diabetes enrolled in the current study, and 36 healthy control. An ophthalmological evaluation was done along with genotyping for the AKR1B1 rs9640883 SNP for all participants. The full demographic data is found in our under-publication study entitled "Thyroid Hormone Levels and the Risk of Diabetic Retinopathy in T2DM".

DNA Extraction

Genomic DNA was extracted using G-spin™ Total DNA Extraction Mini Kit (iNtRON Biotechnology, Korea) as described by the manufacturer. The genotyping of re9640883 SNP on the promoter of the AKR1B1 gene was performed by using a tetra primer-amplification refractory mutation system (ARMS)-PCR.assay. In a single PCR reaction, this approach uses two primer pairs to amplify the two different alleles of a specific SNP. We perform a touchdown PCR technique to reduce nonspecific primer/template binding. The program was as follows: initial denaturation at 95° C for 7 min, 40 cycles of denaturation at 95° C for 30 s and maximum annealing at 65° C decrease gradually every cycle till reached the minimum annealing at 59° C for 40 s and 72° C for extension and final extension for 40 s and 7 min respectively. Primers for the ARMS SNP assays were designed using the SNPgen® tool and listed in Table 1.

Table 1
Primers sequences, PCR conditions and product sizes

Primer name	Primer sequence (5-3)	Tm	Annealing temperature		PCR product size (bp)
AKR IF G	Forward inner primer (G allele): CCGAGGAGCACAAGAAGTGCAG	66	Max 61° C Min. 59° C	Ta=	197 bp (G allele)
AKR IR A	Reverse inner primer (A allele): GGCATCTTCCGTGTCATTTTTCATT	66			142 bp (A allele)
AKR FO	Forward outer primer: TGCTGCACAAGAGTGAATGTTTGAA	66		Ta=	292 bp (two outer primers)
AKR RO	Reverse outer primer: GCCTAGGTGTGTCAGGCTTCAAAGT	66			

Results

The patient group consisted of 134 subjects with T2DM (mean age = 56.26; 72 males, 62 females). One hundred three of them with diabetic retinopathy grouped into NPDR and PDR, and the others (31 patients) were diabetic patients without retinopathy (NDR). The control group was 36 (mean age = 46.64; 22 males, 14 females). BMI, hypertension and smoking status of the patient are 29.68 Kg/m^2, 80 with hypertension, 25 one smoker. Demographic data of the NDR, NPDR and PDR patients are also given in Table 2.

Table 2
Clinical characteristics of controls, diabetic patients and non-diabetic patients

	Control (n=36)	Total T2DM (n= 134)	NDR (n= 31)	NPDR (n=64)	PDR (n=39)
Age	46.64(11.58)	56.26(10.88)	58.52(13.19)	57.30(9.07)	52.97 (10.4)
Gender (years)	22/14	72/62	13/18	36/28	23/16
BMI (kg/m2)	24.01(4.38)	29.68(4.94)	29.46(4.52)	30.10(5.58)	29.49 (4.73)
Hypertension, *n* N/Y	35/1	54/80	14/17	21/43	19/20
Smoking, *n* N/Y	28/8	109/25	26/5	50/14	33/6

Quantitative date are represented as mean ± SD. Qualitative data are represented as counts. BMI=Body mass index; NDR=non-diabetic retinopathy; NPDR= Non proliferative diabetic retinopathy; PDR= proliferative diabetic retinopathy. The result of genotyping the rs9640883 SNP in AKR1B1 with diabetic retinopathy is a GG pattern in all recruited cases with DR or cases without this complication (134 patients) and 36 controls. Figure 1 shows the results of SNP genotyping by tetra primer-ARMS PCR. the band that size 292 bp is to ensure the amplification of the

target region of the AKR1B1 gene while the 197 bp band indicates the presence of GG genotype. No 142 bp band was detected that represents AA genotype or along with 292 bp to represent heterozygote GA genotype.

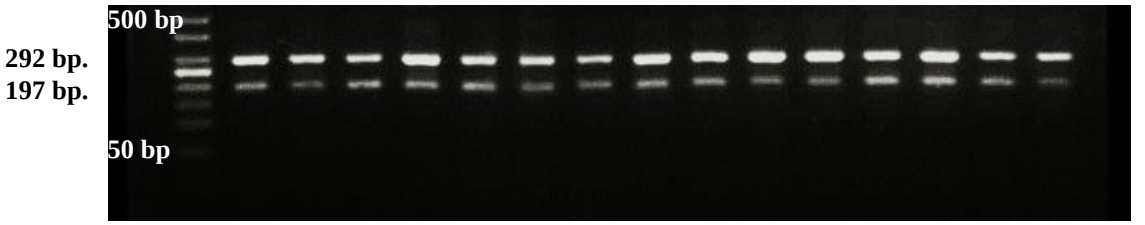

Fig 1. The results of tetra primer-ARMS PCR for genotyping rs9640883, bands with 197 bp indicate GG genotype. The confirmative band's size is 292 bp. (The DNA ladder is 50-500 bp)

Discussion

Numerous research studies have analyzed the relationship between polymorphisms in the AKR1B1 gene, and the risk of developing DR. rs9640883 polymorphism is poorly explored in previous studies. The current study investigated the presence of the rs9640883 variant in the sequence of the AKR1B1 gene. We didn't find the A allele (mutant allele)in the locus of the rs9640883 variant, which might suppose this variant is race dependent. This variant has not been linked to either DR patients or ordinary people in our sample of the Iraqi population. It is important to consider that our population is ethnically different from previous studies on the same variant; furthermore, it is unknown whether this polymorphism has ever been studied in Iraq. In a study by Madsen et al. on three different populations (Africans, Caucasians and Eskimos), the mutant allele frequency was absent in the Eskimos (Madsen et al., 1994).

However, most studies on SNPs and DR have shown conflicting and contradictory results. A study in Egypt shows no association between the SNP at investigation and DR (Shawki et al., 2020) While Kaur et al. found a statistically significant association between the same polymorphism and retinopathy (Kaur et al., n.d.). These studies' contradictory results on rs759853 polymorphism in AKR1B1 gene in different populations and we reviewed here due to there are a limited number of studies on this SNP (rs9640883) moreover to shows that given SNP may largely different from one population to another as it observed in many genetic association studies. Previous studies found that rs9640883 was significantly associated with DR(Abhary et al., 2010; Li et al., 2019; S. I. Mogilevskyy & Bushuieva, 2017). Yet no one found non-association; as mentioned above, the cause (limited studies) and not even a single study in the middle east. A possible reason is that the majority of studies on genetic association with a disease have been performed in Europeans, thus reflecting the incomplete understanding of genetic disease and may result in an inaccurate risk assessment (Sirugo et al., 2019). Another reason, the sample size might be a factor in the discrepancy in findings. So we aimed to investigate the association of the rs9640883 SNP with the development of DR, and the absence of such variant needs more studies to

confirm the DR patients in Iraq all contain the G allele. Also, it is important to investigate other causative genetic variants of the AKR1B1 gene and the susceptibility of DR in our population.

Conclusion

Rs9640883 polymorphism was absent in our samples of DR, and it suggests the presence of the wild type in this locus.

Conflicts of Interest

The authors report no conflicts of interest in this work.

References

Abhary, S., Burdon, K. P., Laurie, K. J., Thorpe, S., Landers, J., Goold, L., Lake, S., Petrovsky, N., & Craig, J. E. (2010). Aldose Reductase Gene Polymorphisms and Diabetic Retinopathy Susceptibility. *Diabetes Care*, *33*(8), 1834–1836. https://doi.org/10.2337/DC09-1893

Brownlee, M. (2005). The Pathobiology of Diabetic ComplicationsA Unifying Mechanism. *Diabetes*, *54*(6), 1615–1625. https://doi.org/10.2337/DIABETES.54.6.1615

Cao, M., Tian, Z., Zhang, L., Liu, R., Guan, Q., & Jiang, J. (2018). Genetic association of AKR1B1 gene polymorphism rs759853 with diabetic retinopathy risk: A meta-analysis. *Gene*, *676*, 73–78. https://doi.org/10.1016/J.GENE.2018.07.014

Demaine, A. (2003). Polymorphisms of the Aldose Reductase Gene and Susceptibility to Diabetic Microvascular Complications. *Current Medicinal Chemistry*, *10*(15), 1389–1398. https://doi.org/10.2174/0929867033457359

Kao, Y. L., Donaghue, K., Chan, A., Knight, J., & Silink, M. (2000). A novel polymorphism in the aldose reductase gene promoter region is strongly associated with diabetic retinopathy in adolescents with type 1 diabetes. *Diabetes*, *48*(6), 1338–1340. https://doi.org/10.2337/DIABETES.48.6.1338

Kaur, N., Practice, V. V.-D. R. and C., & 2016, undefined. (n.d.). Association of aldose reductase gene (AKR1B1) polymorphism with diabetic retinopathy. *Elsevier*. Retrieved May 21, 2022, from https://www.sciencedirect.com/science/article/pii/S0168822716305071

Li, W., Chen, S., Mei, Z., Zhao, F., & Xiang, Y. (2019). Polymorphisms in sorbitol-aldose reductase (Polyol) pathway genes and their influence on risk of diabetic retinopathy among han Chinese. *Medical Science Monitor*, *25*, 7073–7078. https://doi.org/10.12659/MSM.917011

Madsen, H. O., Garred, P., Kurtzhals, J. A. L., Lamm, L. U., Ryder, L. P., Thiel, S., & Svejgaard, A. (1994). A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics*, *40*(1), 37–44.

Mogilevskyy, S. I., & Bushuieva, O. v. (2017). Predicting the development of diabetic retinopathy based on identification of rs759853 and rs9640883 in the AKR1B1 gene. In *Journal of Ophthalmology*.

Mogilevskyy, S., (Ukraine), O. B.-J. of O., & 2017, undefined. (n.d.). Predicting the development of diabetic retinopathy based on identification of rs759853 and

rs9640883 in the AKR1B1 gene. *Ozhurnal.Com*. Retrieved May 21, 2022, from http://www.ozhurnal.com/sites/default/files/2017-4-1.pdf

Pinazo-Durán, M. D., Zanón-Moreno, V., Lleó-Perez, A., García-Medina, J. J., Galbis-Estrada, C., Roig-Revert, M. J., Marco-Ramírez, C., López-Gálvez, M., Dolz-Marco, R., Duarte, L., Campos Borges, C., Salgado-Borges, J., & Gallego-Pinazo, R. (2016). Genetic systems for a new approach to risk of progression of diabetic retinopathy. *Archivos de La Sociedad Española de Oftalmología (English Edition)*, *91*(5), 209–216. https://doi.org/10.1016/J.OFTALE.2016.01.016

Priščáková, P., Minárik, G., & Repiská, V. (2016). Candidate gene studies of diabetic retinopathy in human. *Molecular Biology Reports*, *43*(12), 1327–1345. https://doi.org/10.1007/S11033-016-4075-Y

Romero-Aroca, P., Baget-Bernaldiz, M., Pareja-Rios, A., Lopez-Galvez, M., Navarro-Gil, R., & Verges, R. (2016). Diabetic Macular Edema Pathophysiology: Vasogenic versus Inflammatory. *Journal of Diabetes Research*, *2016*. https://doi.org/10.1155/2016/2156273

Shawki, H. A., Elzehery, R., Abo-hashem, E. M., Shahin, M., & Youssef, M. M. (2020). Gene polymorphism of C106T "rs759853" is not associated with diabetic retinopathy in Egyptian patients with type 2 diabetes mellitus. *Gene Reports*, *21*. https://doi.org/10.1016/j.genrep.2020.100865

Sirugo, G., Williams, S. M., & Tishkoff, S. A. (2019). The missing diversity in human genetic studies. *Cell*, *177*(1), 26–31.

Ting, D. S. W., Cheung, G. C. M., & Wong, T. Y. (2016). Diabetic retinopathy: global prevalence, major risk factors, screening practices and public health challenges: a review. *Clinical & Experimental Ophthalmology*, *44*(4), 260–277. https://doi.org/10.1111/CEO.12696

Wang, Y., Ng, M. C. Y., Lee, S.-C., So, W.-Y., Tong, P. C. Y., Cockram, C. S., Critchley, J. A. J. H., & Chan, J. C. N. (2003). Phenotypic heterogeneity and associations of two aldose reductase gene polymorphisms with nephropathy and retinopathy in type 2 diabetes. *Am Diabetes Assoc*. https://diabetesjournals.org/care/article-abstract/26/8/2410/22913