MANSOURA JOURNAL OF CHEMISTRY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

An Association of Pro12Ala Polymorphism of the PPAR-γ Gene with Stroke in the Egyptian Population

Mariam Saad[1*], Afaf M. ElSaid[2], Wessam Mustafa[3], Omali Y. El-khawaga[1]

[a] *Biochemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt.*

[b] *Genetic unit, department of pediatrics, Faculty of Medicine, Mansoura University, Mansoura, 35516, Egypt.*

[c] *Neurology Department, Mansoura University Hospital, Mansoura, 35516, Egypt.*

**Corresponding author: Mariam Saad, Biochemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt. Email: mariemsayd@gmail.com, +201093840227*

Received:19/10/2023
Accepted: 13/12/2023

Abstract: The current research aims to investigate the association between PPAR-γ genetic polymorphism at the rs1801282 loci and ischemic stroke risk in the Egyptian population. Method: The study included 100 individuals diagnosed with ischemic stroke and 150 age- and sex-matched healthy controls. DNA was extracted from peripheral blood samples prior to the detection of SNP genotyping via ARMS-PCR. The study found that CG and GG genotypes (CG vs. CC+GG) were more common in stroke groups compared to the control group (P = 0.001), which meant that people with these genotypes were more likely to have a stroke. However, there were no significant differences between the two groups of cases and controls for the recessive model (P > 0.05). No significant association was found regarding blood pressure, TG, or TC among stroke patients. Conclusion: The common polymorphism in PPARγ, rs1801282 C>G, increases the susceptibility to ischemic stroke in the Egyptian population. Further study of the association between the SNP and ischemic stroke is needed in larger studies.

Key words: single nucleotide marker, PPAR-γ.

Introduction:

According to the Global Burden of Disease (GBD) 2019, stroke is the world's second leading cause of death and disability combined. [1] [2]. Males account for 77.0 million disability-adjusted life-years lost (DALYs) due to stroke, while females account for 66.0 million. The lifetime risk of stroke has increased by 50% over the last 20 years, now affecting one in four people. There are no significant sex differences in stroke-related deaths [3]. The two broad stroke classes, hemorrhage and ischemia, are diametrically opposed conditions: Hemorrhage stroke is defined by an excess of blood within the closed cranial cavity, whereas ischemic stroke is defined by an insufficient supply of oxygen and nutrients to a portion of the brain. [4]. It is known that conventional risk factors for stroke include high blood pressure, atrial fibrillation, smoking, high cholesterol, diabetes, and obesity. However, these factors do not fully account for the occurrence of strokes, and despite extensive investigations, approximately 30% of stroke cases remain of unknown origin. [5]. Figuring out genetic variants associated with the risk of ischemic stroke could aid in the pathogenesis of the disease and lead to new approaches for the management and prevention of this complex disease. [6]. PPARs (PPAR-α, PPAR-δ, and PPAR-γ) are transcription factors which belong to the nuclear hormone receptor superfamily and

regulate various genes involved in inflammation, glucose and lipid metabolism, adipogenesis, and carcinogenesis [3], [7]. The PPARG gene on chromosome 3p25.2 in humans encodes the nuclear peroxisome proliferator activated receptor-γ (PPAR-γ), a crucial protein involved in numerous processes. Its gene variability may serve as a predictive genetic marker for IS. [8]. Numerous instances of single-nucleotide polymorphisms (SNPs) in the PPAR- gene have attracted attention and influenced PPAR- expression or activity. [9]. Of these SNPs, rs1801282 (Pro12Ala) found in the PPAR- gene has been extensively studied in terms of its associations with atherosclerosis and stroke. Despite this, some studies have found that carriers have a lower risk of atherosclerosis. [7] [10] [11]. As a result, the current study sought to examine the relationship between PPAR- genetic polymorphism at the rs1801282 loci and ischemic stroke risk in the Egyptian population.

2. Materials and methods

The proposal was submitted to the Mansoura Faculty of Medicine Institutional Research Board (MFM-IRB) for approval (ethical code: MS.22.05.2009, date: 31/5/2022). Patients and control participants signed informed consent forms. They were given a unique code to safeguard their privacy. Our study included 100 individuals diagnosed with ischemic stroke and 150 age- and sex matched healthy controls. Cardiologists diagnosed hypertension in patients based on the guidelines established by the International Society of Hypertension [systolic blood pressure (SBP) ≥140 mmHg and diastolic blood pressure (DBP) ≥ 90 mmHg] [15]. Healthy controls will be chosen based on their lack of tumors, trauma, and history of transient ischemic attacks. Nobody in the control group smokes. There were no unforeseen risks during the research. Participant data were collected between May 2022 and March 2023 from Mansoura University Hospital, Daqhlya, Egypt. Waste materials were burned. The molecular testing for genetic alterations was done in the lab.

Blood sampling

For the investigation of genetic mutations and hematological parameters, samples were collected by drawing 3 ml of the blood from all participants into (EDTA) tubes. After being collected, each sample was kept at -20 20°C. They were placed at room temperature before the technique to be used for DNA extraction. To find gene polymorphism, then subjected to PCR analysis and gel electrophoresis.

Extraction of Genomic DNA

Leukocytes were isolated from 2 mL of blood samples, and genetic material was subsequently extracted using the common techniques of a commercial Easy Pure® DNA purification kit (Transe GEN Easy Pure® Cat. No. EE121-01). DNA was measured via UV light absorption spectrometric at 260 nm wavelength. Each specimen was set at 25 ng/L in preparation for genotyping.

PCR amplification of *PPAR-γ (Pro12Ala)* rs1801282: Two tubes were used for every subject. Each PCR reaction mixture was performed in an overall volume of 24 µl including 4 µl of forwarding control and 4 µl of reverse primer (RG) or 4 µl of reverse primer (RC), and 12 µl of master mix (COSMO RED Master Mix (W10203001), willow fort). The PCR assay conditions were initial denaturation 94°C, 5

minutes for 1 cycle; 36 cycle including denaturation at 94°C, 30 second; annealing 65°C, 30 second; 72°C for extension, for 50 seconds; and final extension at 72°C, for 5 minutes, in 1cycle; then soak at 4°C. The products of PCR amplification were electrophoresed on 2.5 % agarose gel. They were visualized under UV transillumination. The procedure rendered three bands (internal control at 455, CC genotype Proline/Proline at 211 and GG genotype Alanine/Alanine at 288 bp) [12].

Table (1). The primer sequences which were used for *PPAR-γ* rs1801282.

Mutation	Primer sequence	Size (bp)
***PPAR-γ* rs1801282**	**FC:**5´-AAC TTT TTG TCA CAG CTG GCT CCT AAT A-3´	**288**
	R (G): 5´- GTA TCA GTG AAG GAA TCG CTT TCA GC -3´	
	FC: 5´- GAA ACT CTG GGA GAT TCT CCT ATT GTC C -3´	**221**
	R(C): 5´ -CAA CGA GCT AAG CAT TAA AAT ACT GGA-3´	

Table (2): Comparison of demographic characteristics among studied groups:

	Control n=150	Stroke cases n=100	Test (p)	*P*
Age (years)	64 (19-88)	64 (19-88)	T=0.204	0.839
SEX (male/female)	79/71	60/40	X2=1.307	0.253
SBP (mmg)	124 (100- 198)	130 (90- 190)	T=2.413	**0.017**
DBP (mmg)	80 (58- 89)	80 (50-100)	T=3.740	**<0.001**

P, probability; p<0.05 is significant; T, student test; X2, chi square test.

Biochemical analysis:

After an 8-hour fast, blood samples were obtained in the morning and used to calculate serum lipid levels. Enzymatic techniques were used to determine the amounts of TC and TG in samples using commercially available kits; TC, (BioMed- Cholesterol- LS (#CHO104090), Egypt), TG (Biomed- Triglycerides L.S (#TG117090), (Cairo, Egypt).).

Statistical analysis:

The data collected were analyzed and lobulated using the SPSS software package (IBM Corp. 2017. windows SPSS Statistics, Version 25.0. Armonk, NY: IBM Corp.). According to the demographic and clinical characteristics of the study population, categorical variables, including gender, are presented as frequencies with percentages.

3.Results:

This study was conducted on 100 cases of stroke. Their mean age was 61.9, which ranged from 19 to 88 years. They were 60% males and 40% females. In addition to 150 healthy control subjects, The cases and control are age and sex-matched; there is no statistically significant difference between both groups regarding age (P = 0.839) or sex (P = 0.253). Blood pressure and laboratory investigation between patients and healthy controls showed that stroke cases had significantly higher systolic blood pressure (SBP) (P =0.017) and significantly lower diastolic blood pressure (DBP) (P <0.001, for each) when compared to the control healthy group. Table (3).

Distribution of PPAR-γ gene polymorphism in Controls compared to stroke patients.

Applying Hardy Weinberg equation, we calculated the expected count and revealed that all studied SNP's genotypes in the control group as well as in the cases' groups were in HW equilibrium. PPAR-γ gene polymorphism was assessed among all studied subjects. Table (4) shows a very statistically significant difference in PPAR-γ genotype polymorphisms from controls. The CC homozygous genotype was observed in patients (21%) and (58.7%) in the healthy controls; the CG heterozygous genotype was lower in patients (39.3%) than in the control group (59%). The GG homozygous genotype was higher in the patient (4%) than in the control (2%).

GC genotype (Odds ratio [OR], 5.327 ; Probability (P=<0.001)), GG genotype (Odds ratio [OR], 5.587; Probability (P=0.032)), (CG) over dominant (Odds ratio [OR], 4.627; Probability (P=<0.001)), (CG+GG) dominant models ([OR], 5.339; P=<0.001), G-Allele allelic model (Odds ratio [OR], 2.565; Probability (P=<0.001)) showed higher frequency among stroke group when compared to control group, with risk to develop stroke, while Recessive model showed no significant differences between cases and controls (P>0.05). **Figure (1)**

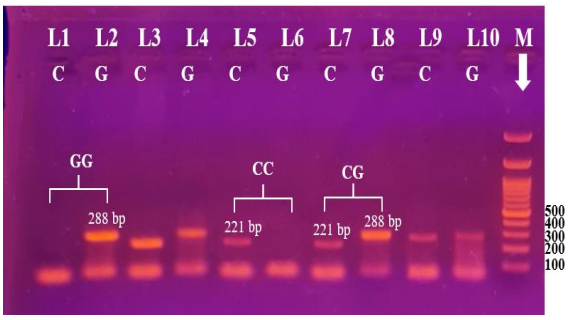

Figure (1): Gel electrophoresis of the PCR product of PPAR-γ by ARMS PCR, when each of the two lanes represents one participant. M stands for DNA marker (100 bp). Specific 221 bp bands represent the C allele, and specific 288 bp bands represent the G allele. Lanes 1 and 2 indicate the GG homozygous genotype, whereas the G allele appears in lane 2 and the C allele is absent from lane 1. Lanes (5 and 6) are CC homozygous, where the C allele appears at lane (5) at 221 bp and the G allele is absent from lane (6). Lanes (3, 4, 7, 8, 9, and 10) indicate CG heterozygous, where the C allele appears at lanes (3, 7, and 9) at 221 bp and the G allele appears at lanes (4, 8, and 10) at 288 bp

Association of PPAR-γ gene genotype polymorphisms with other parameters in stroke patients:

We investigated the association of age, sex, and blood pressure with the rs1801282 PPAR-γ mutation. No significant association was found regarding PPAR-γ with gender or age among all studied cases (P > 0.05). Also, no significant association was found regarding blood pressure, TG, or TC among stroke patients (P > 0.05). Table (5)

Table (3): Association of PPAR-γ gene genotype polymorphisms with other parameters in stroke patients:

		CG N=75	CG+GG n=79	CC n=21	GG n=4	Test 2	P2	Test (1)	p1
Sex n (%)	male	44(59.5%)	46 (58.2%)	14 (66.7%)	2(50%)	X2=0.552	0.759	X2=0.492,	0.483
	female	30(40.5%)	33 (41.8%)	7 (33.3%)	2(50%)				
Agemedian (range)		63(19.0 - 88.0)	63.0 (19.0 –88.0)	65.0 (41.0 –83.0)	62.5 (51.0 - 75.0)	F=0.082	0.922	T=0.298	0.767
SBP (mmg)		130 (90 - 190)	130 (90 – 190)	130 (100 -150)	130 (100-140)	F=0.350	0.706	T=0.322	0.749
DBP (mmg)		80(60 - 100)	80(50– 100)	80(60 – 90)	75(50 - 80)	F=1.990	0.142	T=0.032	0.974
Triglyceride (mg/dl)		132(26- 597)	131(26 – 887)	109(62 –276)	110(77-131)	H=2.751	0.253	U=1001.5	0.145
TC (mg/dl)		169.5(90 - 302)	171(90– 302)	148(72 –240)	178(157195)	F=1.471	0.235	T=1.764	0.081

P: probability, p < 0.05 = significant T, student test; X2, chi square test; U, Mann–Whitney test.

Discussion:

Approximately 85% of all stroke cases are caused by ischemia, which is the result of cerebral blood artery blockage primarily due to emboli with intracranial stenosis or thrombosis concomitant with blood clot formation [13]. With an increase in the ageing population, the number of ischemic stroke (IS) patients has been increasing in recent years and is predicted to continue, which brings a substantial economic and social burden to the public health system. Thrombosis caused by atherosclerosis and inflammatory injury of the vascular endothelium is one of the most direct causes of IS and is significantly associated with aberrant expression or dysfunction of the PPAR family [14]. PPAR-is a nuclear receptor superfamily member and a ligand-activated transcription factor that is widely expressed in adipose cells and tissues and plays an important role in the regulation of adipogenesis metabolism, insulin sensitivity, energy balance, inflammation, angiogenesis, microvascular lesions, and atherosclerosis. [15].

Over the past few decades, it has been discovered that PPAR-agonists, which are known to lower inflammation in a number of IS models, also reduce proinflammatory mediators during the course of IS development. [16]. PPAR-1agonists, on the other hand, have been shown to reduce the incidence of stroke recurrence and total occurrences of cardiovascular mortality or stroke by preventing the development of

arteriosclerosis. [17], this suggests that PPAR- may play a protective role in vascular aging. Furthermore, PPAR- activation has been shown to increase angiogenesis and migration of human microvascular endothelial cells in the cerebrovascular system via fibroblast growth factor production, improve vascular recanalization ability, and improve nerve healing after stroke. [18]. All of these lines of evidence point to PPAR- being involved in the pathophysiology of IS. However, the underlying pathophysiology of PPAR- in the IS is unknown. The present study aimed to investigate the relationship between the Pro12Ala polymorphism of the PPAR-γ gene and stroke disease severity in a sample of Egyptian individuals (healthy and patient subjects). The current comparison among cases with stroke showed a very strong and significant prevalence of the CG genotype and G allele in comparison with controls, as well as the dominant and over-dominant models ($P < 0.001$ for each). In addition, the CG heterozygous genotype investigates a significant increase in stroke patients compared to healthy individuals ($P < 0.03$). To our knowledge, this is the first study to suggest that the PPAR-γ genetic variant rs1801282 plays a considerable role in the etiology of ischemic stroke in the Egyptian population. A previous study of Chinese ethnicity investigated a similar observation of the dominant model as well as the heterozygous model ($P< 0.05$ for each) [19].

Many researches have been undertaken to investigate the link between PPAR- polymorphisms and the risk of essential hypertension (EH) in various populations. [20]. Previous research on the connection of PPAR-Pro12Ala polymorphism with increased hypertension (EH) risk has been considerable, however the findings remain contentious. [21]. The current result showed no significant difference between EH in SBP and DBP in stroke patients ($P > 0.05$). A similar result was found by Horiki et al., as they did not find an association between the PPAR-γ Pro12Ala polymorphism and EH [22]. The common polymorphism rs1801282 (Pro12Ala) is associated with greater insulin sensitivity [23]. Because the PPARG-rs1801282 polymorphism reduces transcriptional activity, it may reduce insulin signaling and restrict the function of pancreatic islet B cells, eventually leading to diabetes mellitus. [24], [25]. PPARG-rs1801282 expression has been linked to decreased lipoprotein lipase activity in recent years. [26] and have an effect on triglyceride removal, resulting in atherosclerosis and dyslipidemia. This research was supported by a previous study. [27]. However, In the present study, using patients with the PPARG-rs1801282, we found no associations among Pro12Ala SNP and dyslipidemia or diabetes. Another finding showed similar results to the current study [28]. These disparities may be caused by genetic heterogeneity resulting from ethnic differences. The sample size, research methodology, or patient selection criteria might be additional causes of the inconsistent outcomes. More research is necessary to determine the exact processes behind these discoveries. In conclusion, the common polymorphism in PPARG, rs1801282 C>G, increases the susceptibility to IS in the Egyptian population. Further study of the association between the SNP and IS is needed in larger studies.

4.Reference:

1 V. L. Feigin *et al* (2022) “World Stroke Organization (WSO): Global Stroke Fact Sheet,” *International Journal of Stroke*, vol. **17**, no. 1, pp. 18–29, 2022, doi: 10.1177/17474930211065917.

2 V. L. Feigin *et al.*, (2019)“Global, regional, and national burden of stroke and its risk factors, 1990-2019: A systematic analysis for the Global Burden of Disease Study,” The Lancet Neurology, vol. **20**, no. 10, pp. 1–26, 2021, doi: 10.1016/S1474-4422(21)00252-0.

3 M. O. Owolabi *et al.*, (2022) “Primary stroke prevention worldwide: translating evidence into action,” The Lancet Public Health, vol. **7**, no. 1, pp. e74–e85, doi: 10.1016/S2468-2667(21)00230-9.

4 L. R. Caplan, “Intracranial branch atheromatous disease,” Neurology, vol. **39**, no. 9, pp. 1246 LP – 1246, Sep. 1989, doi: 10.1212/WNL.39.9.1246.

5 S. Yaghi, R. A. Bernstein, R. Passman, P. M. Okin, and K. L. Furie, (2017), “Cryptogenic Stroke: Research and Practice,” Circulation Research, vol. **120**, no. 3, pp. 527–540, doi: 10.1161/CIRCRESAHA.116.308447.

6 L. Xia *et al.*, (2018), “Combined analysis of interleukin-10 gene polymorphisms and protein expression in children with cerebral palsy,” Frontiers in Neurology, vol. **9**, no. MAR, Mar. doi: 10.3389/fneur.2018.00182.

7 V. Paracchini, P. Pedotti, and E. Taioli, (2005) “Genetics of leptin and obesity: A HuGE review,” *American Journal of Epidemiology*, vol. **162**, no. 2, pp. 101–114, doi: 10.1093/aje/kwi174.

8 M. E. Greene *et al.*, (1995) “isolation of the human peroxisome proliferator activated receptor gamma cDNA: Expression in hematopoietic cells and chromosomal mapping,” Gene Expression, vol. **4**, no. 4–5, pp. 281–299,

9 C. Janani and B. D. Ranjitha Kumari, (2015), “PPAR gamma gene - A review,” Diabetes and Metabolic Syndrome: Clinical Research and Reviews, vol. **9**, no. 1, pp. 46–50, doi: 10.1016/j.dsx.2014.09.015.

10 G. Xie *et al.*, (2013) “Relationship of serum interleukin-10 and its genetic variations with ischemic stroke in a Chinese general population.,” PloS one, vol. **8**, no. 9, doi: 10.1371/*journal.pone*.0074126.

11 P. Wang *et al.*, (2015), “Association between peroxisome proliferator-activated receptor gamma gene polymorphisms and atherosclerotic diseases: A meta-analysis of case-control studies,” *Journal of Atherosclerosis and Thrombosis*, vol. **22**, no. 9, pp. 912–925, doi: 10.5551/jat.26138.

12 R. M. Shawky, T. M. Kamal, S. Raafat, and G. H. El Nady (2018), “Genotyping of PPAR-γ gene polymorphism in Egyptian neonates affected with sepsis disease and its severity,” *Egyptian Journal of Medical Human Genetics*, vol. **19**, no. 3, pp. 215–220, doi: 10.1016/j.ejmhg.2017.09.005.

13 M. Zhou *et al.*, (2017) “Mortality, morbidity, and risk factors in China and its provinces, 1990–2017: a systematic analysis for the Global Burden of Disease Study,” The Lancet, vol. **394**, no. 10204, pp. 1145–1158, 2019, doi: 10.1016/S0140-6736(19)30427-1.

14 F. Cheng, X. M. Si, G. L. Yang, and L. Zhou, (2021), “Relationship between PPAR-γ gene polymorphisms and ischemic stroke risk: A meta-analysis,” Brain and Behavior, vol. **11**, no. 12, doi: 10.1002/brb3.2434.

15 R. M. Rocha, G. B. Barra, É. C. C. C. Rosa, É. C. Garcia, A. A. Amato, and M. F. Azevedo, (2015), “Prevalence of the rs1801282 single nucleotide polymorphism of the PPARG gene in patients with metabolic syndrome,” Archives of Endocrinology and Metabolism, vol. **59**, no. 4, pp. 297–302, Aug. doi: 10.1590/2359-3997000000086.

16 M. Gliem, L. Klotz, N. Van Rooijen, H. P. Hartung, and S. Jander, (2015), “Hyperglycemia and PPARγ antagonistically influence macrophage polarization and infarct healing after ischemic stroke,” Stroke, vol. **46**, no. 10, pp. 2935–2942, doi: 10.1161/STROKEAHA.115.010557.

17 J. Liu and L.-N. Wang, (2019), “Peroxisome proliferator-activated receptor gamma agonists for preventing recurrent stroke and other vascular events in people with stroke or transient ischaemic attack,” Cochrane Database of Systematic Reviews, vol. **2019**, no. 10, doi: 10.1002/14651858.cd010693.pub5.

18 W. Huang *et al.*,(2019), “Fibroblast growth factor 21 enhances angiogenesis and wound healing of human brain microvascular endothelial cells by activating PPARγ,” *Journal of Pharmacological Sciences*, vol. **140**, no. 2, pp. 120–127, doi: 10.1016/j.jphs.2019.03.010.

19 Y. Z. Wang, H. Y. Zhang, F. Liu, L. Li, S. M. Deng, and Z. Y. He, (2019), "Association between PPARG genetic polymorphisms and ischemic stroke risk in a northern Chinese Han population: A case-control study," Neural Regeneration Research, vol. **14**, no. 11, pp. 1986–1993, Jul. doi: 10.4103/1673-5374.259621.

20 G. Cai, X. Zhang, W. Weng, G. Shi, S. Xue, and B. Zhang, (2017), "Associations between PPARG polymorphisms and the risk of essential hypertension," *PLoS ONE*, vol. **12**, no. 7, doi: 10.1371/*journal.pone*.0181644.

21 A. Bener, S. Darwish, A. O. A. A. Al-Hamaq, R. M. Mohammad, and M. T. Yousafzai, (2013), "Association of PPARγ2 gene variant Pro12Ala polymorphism with hypertension and obesity in the aboriginal Qatari population known for being consanguineous," Application of Clinical Genetics, vol. **6**, pp. 103–111, doi: 10.2147/TACG.S49875.

22 M. Horiki *et al.*, (2004) "Association of Pro12Ala polymorphism of PPARγ gene with insulin resistance and related diseases," Diabetes Research and Clinical Practice, vol. **66**, no. SUPPL. doi: 10.1016/j.diabres.2003.09.023.

23 Y. S.M. *et al.*, (2014) "Combined effects of the C161T and Pro12Ala PPAR(gamma)2 gene variants with insulin resistance on metabolic syndrome: A case-control study of a central tunisian population," *Journal of Molecular Neuroscience*, vol. **52**, no. 4. pp. 487–492,

24 N. B. Kasim, H. Z. Huri, S. R. Vethakkan, L. Ibrahim, and B. M. Abdullah, (2016), "Genetic polymorphisms associated with overweight and obesity in uncontrolled Type 2 diabetes mellitus," Biomarkers in Medicine, vol. **10**, no. 4, pp. 403–415, doi: 10.2217/bmm-2015-0037.

25 S. S. Priya, R. Sankaran, S. Ramalingam, T. Sairam, and L. S. Somasundaram,(2016), "Genotype Phenotype Correlation of Genetic Polymorphism of PPAR Gamma Gene and Therapeutic Response to Pioglitazone in Type 2 Diabetes Mellitus- A Pilot Study.," *Journal of clinical and diagnostic research : JCDR*, vol. **10**, no. 2, pp. FC11-4, Feb. doi: 10.7860/JCDR/2016/16494.7331.

26 H. J. Xie *et al.*, (2014), "Analysis on the association between PPARα/γ polymorphisms and lipoprotein (a) in a Chinese Han population.," Molecular genetics and genomics : MGG, vol. **289**, no. 5, pp. 981–987, doi: 10.1007/s00438-014-0866-9.

27 Y. Z. Wang, H. Y. Zhang, F. Liu, L. Li, S. M. Deng, and Z. Y. He, (2019), "Association between PPARG genetic polymorphisms and ischemic stroke risk in a northern Chinese Han population: A case-control study," Neural Regen Res, vol. **14**, no. 11, pp. 1986–1993, doi: 10.4103/1673-5374.259621.

28 B. C. Lee, H. Lee, and J.-H. Chung, (2006), "Peroxisome proliferator-activated receptor-γ2 Pro12Ala polymorphism is associated with reduced risk for ischemic stroke with type 2 diabetes," Neuroscience Letters, vol. **410**, no. 2, pp. 141–145, doi: https://doi.org/10.1016/j.neulet.2006.08.024 .