Molecular Study of Genomics Mutations in RNF213,ACTA2,GUCY1A3 Genes in patients Moyamoya Syndrome in Human

Shahin Asadi[1*] (student of Molecular Genetics)

Mahsa Jamali[2] (student of Molecular Genetics)

Samaneh Sadeh Dell[2] (student of Molecular Genetics)

Saeedeh Habibi[3] (student of Molecular Genetics)

Young Researchers and Elite Club, Tabriz,Branch,Islamic Azad University,Tabriz,Iran.
Tell;+989143188935,+989379923364

Email;shahin.asadi1985@gmail.com

[*]Author; Shahin Asadi-Molecular Genetics-IRAN-TABRIZ. Director of the Division of Medical Genetics and Molecular Genetics Research.

Abstract

In this study we have analyzed 50 people. 20 patients Moyamoya disease and 30 persons control group.The genes RNF213 on chromosome 17q25 ,ACTA2 on chromosome 10q23.3 ,GUCY1A3 on chromosome 4q32 , analyzed in terms of genetic mutations made. In this study, people who have genetic mutations were targeted, with nervous disorders, Moyamoya disease. In fact, of all people with Moyamoya disease. 20 patients Moyamoya disease had a genetic mutations in the genes RNF213 on chromosome 17q25 ,ACTA2 on chromosome 10q23.3 ,GUCY1A3 on chromosome 4q32 Moyamoya disease. Any genetic mutations in the target genes control group, did not show.

Key Words: Genetic study, Moyamoya disease,Mutations The genes RNF213,ACTA2,GUCY1A3.

Introducation

Moyamoya disease is a disease in which certain arteries in the brain are constricted. Blood flow is blocked by the constriction, and also by blood clots (thrombosis).[1]

A collateral circulation develops around the blocked vessels to compensate for the blockage, but the collateral vessels are small, weak, and prone to bleeding, aneurysm and thrombosis. On conventional X-ray angiography, these collateral vessels have the appearance of a "puff of smoke" (described as "もやもや (moyamoya)" in Japanese).[1]

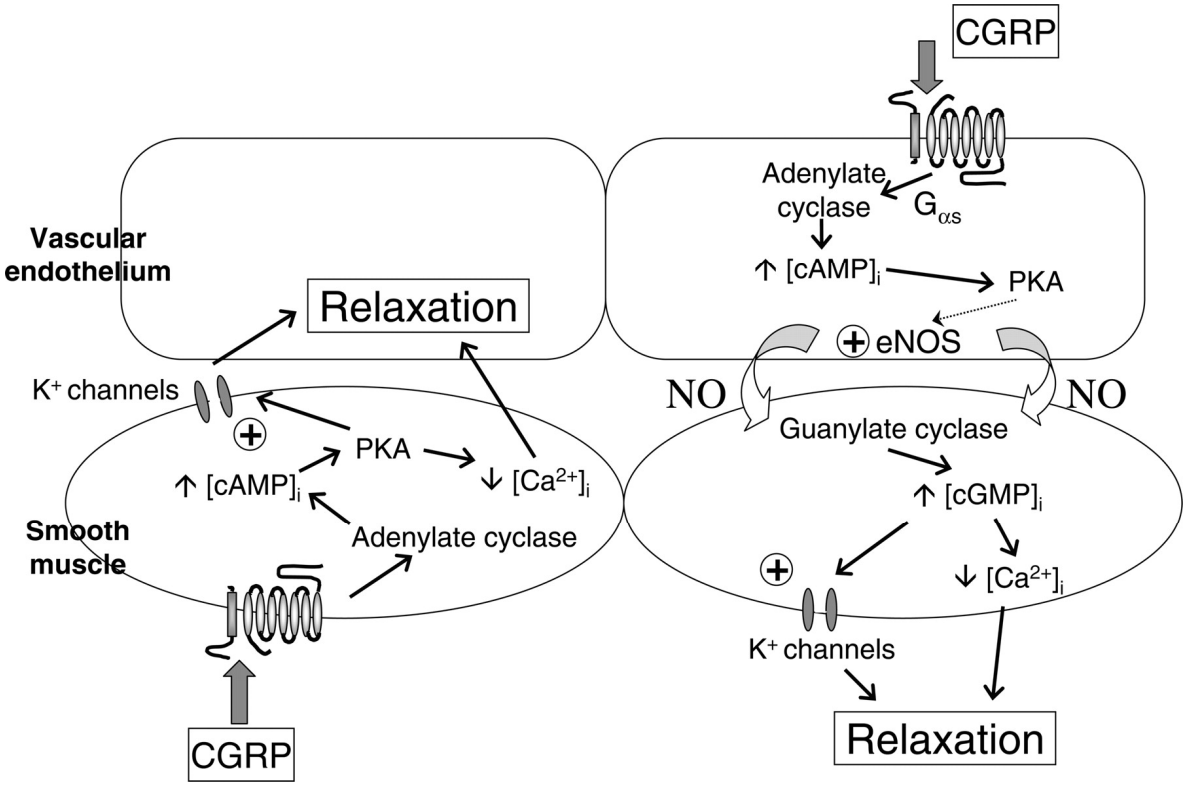


Figure 1: Schematic view of gene molecular the cycle of CGRP.

About 10% of cases of moyamoya disease are familial, and some cases result from specific genetic mutations. Susceptibility to moyamoya disease-2 (MYMY2; 607151) is caused by variation in the RNF213 gene (613768) on chromosome 17q25. Moyamoya disease-5 (MYMY5; 614042) is caused by mutation in the ACTA2 gene (102620) on chromosome 10q23.3; and moyamoya disease-6 with achalasia (MYMY6; 615750) is caused by mutation in the GUCY1A3 gene (139396) on chromosome 4q32. Loci for the disorder have been mapped to chromosome 3p (MYMY1) and chromosome 8q23 (MYMY3; 608796). See also MYMY4 (300845), an X-linked recessive syndromic disorder characterized by moyamoya disease, short stature, hypergonadotropic hypogonadism, and facial dysmorphism. and linked to q25.3, on chromosome 17". (Online Mendelian Inheritance in Man, omim.org/entry/252350).

In Japan the overall incidence is higher (0.35 per 100,000).[2] In North America, women in the third or fourth decade of life are most often affected, but the condition may also occur during infancy or childhood. These women frequently experience transient ischaemic attacks (TIA), cerebral hemorrhage, or may not experience any symptoms at all. They have a higher risk of recurrent stroke and may be experiencing a distinct underlying pathophysiology compared to patients from Japan.[3]

Moyamoya disease can be either congenital or acquired. Patients with Down syndrome, sickle cell anemia, neurofibromatosis type 1, congenital heart disease, fibromuscular dysplasia, activated protein C resistance, or head trauma can develop moyamoya malformations.[4] It is more common in women than in men, although about a third of those affected are male.[5]

The disease moyamoya, which is a Japanese mimetic word, gets its characteristic name due to the appearance of smoke on relevant angiographs resultant from the tangle of tiny vessels in response to stenosis. This makes the blood leak out of the arteries, causing pressure to the brain and subsequent headaches. The pathogenesis of moyamoya disease is unknown, although the gene ring finger protein 213 (RNF213) has been implicated.[6]

Once it begins, the vascular occlusion tends to continue despite any known medical management. In some people this leads to transient ischemic attacks or repeated strokes with severe functional impairment or even death. In others, the blockage may not cause any symptoms.[*citation needed*]

Materials and Methods

In this study,20 patients with Moyamoya disease.and 30 persons control group were studied. Peripheral blood samples from patients and parents with written permission control was prepared.After separation of serum, using Real Time-PCR technique of tRNA molecules were collected. To isolate Neuroglial cells erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solutionin ratio of 1to5with the peripheral blood of patients and controls were mixed.After 60 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 14 min at 400 Gera. The cells ediment with PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrateratio of 1to2onficole (Ficol) was pouredin the 480G was centrifuged for 34 minutes.Mono nuclear Neuroglial cells also are included,has alower density than ficole and soon which they are

based.The remaining erythrocytes has a molecular weight greater than fico leand depositedin test tubes.

The supernatant, which contained the mono nuclear cells was removed, and the 400 Gera was centrifugedfor 12 minutes. Finally, the sediment cell, the antibody and Neuroglial cells was added after 34 minutes incubation at 5 °C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the column LSMACSS pam Stem cell culture medium containing the transcription genes RNF213,ACTA2,GUCY1A3,and were kept.

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose,approximately 4-5 × 10^3 Neuroglial cells were transfer red to1.5ml Eppendorf tube and then was centrifuged at 2000 rpm for 7minutes at time. Remove the supernatant culture medium and there maining sediment, 100µl of PBS buffer was added. After adding 5-10µl PE monoclonal anti body to the cell suspension for 60 min at 4°C, incubated and readimme diately by flow cytometry. For example,rather than control anti body Neuroglial cells PE,IgG1 negative control solution was used.

Total mRNA extraction proced urein cludes:
1) 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200µl chloroform solution to target mix, then transfer the micro tubes were added,and the shaker well was mixed for 15 seconds. The present mix for 4 minutes at room temperature and then incubated for 20 min at 4°C an was centrifuged at 13200 rpm era. Remove the upper phase product were transfer reductase new microtube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hours at -20°C were incubated.

2)Then for 45min at 4°C an was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4°C an by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placed at room temperature until completely dry deposition. The precipitate was dissolved in 20µl sterile water and at a later stage, the concentration of extracted mRNA was determined.

To assessment the quality of mi-RNAs,the RT-PCR technique was used. The cDNA synthesis inreverse transcription reaction (RT) kit (Fermentas K1622) and 1µl oligoprimers 18 (dT) was performed. Following the PCR reaction 2µM dNTP, 1µg cDNA,FermentasPCR buffer 1X, 0 / 75µM MgCl2, 1.25 U / µL Tag DN Aat 95°C for 4 min, 95°C for 30s, annealing temperature 58°C for 30s, and72 °C for30 seconds, 35 cycleswere performed. Then 1.5% agarose gel,the PCR product was dumped in wells after electrophores is with ethidium bromide staining and color were evaluated.

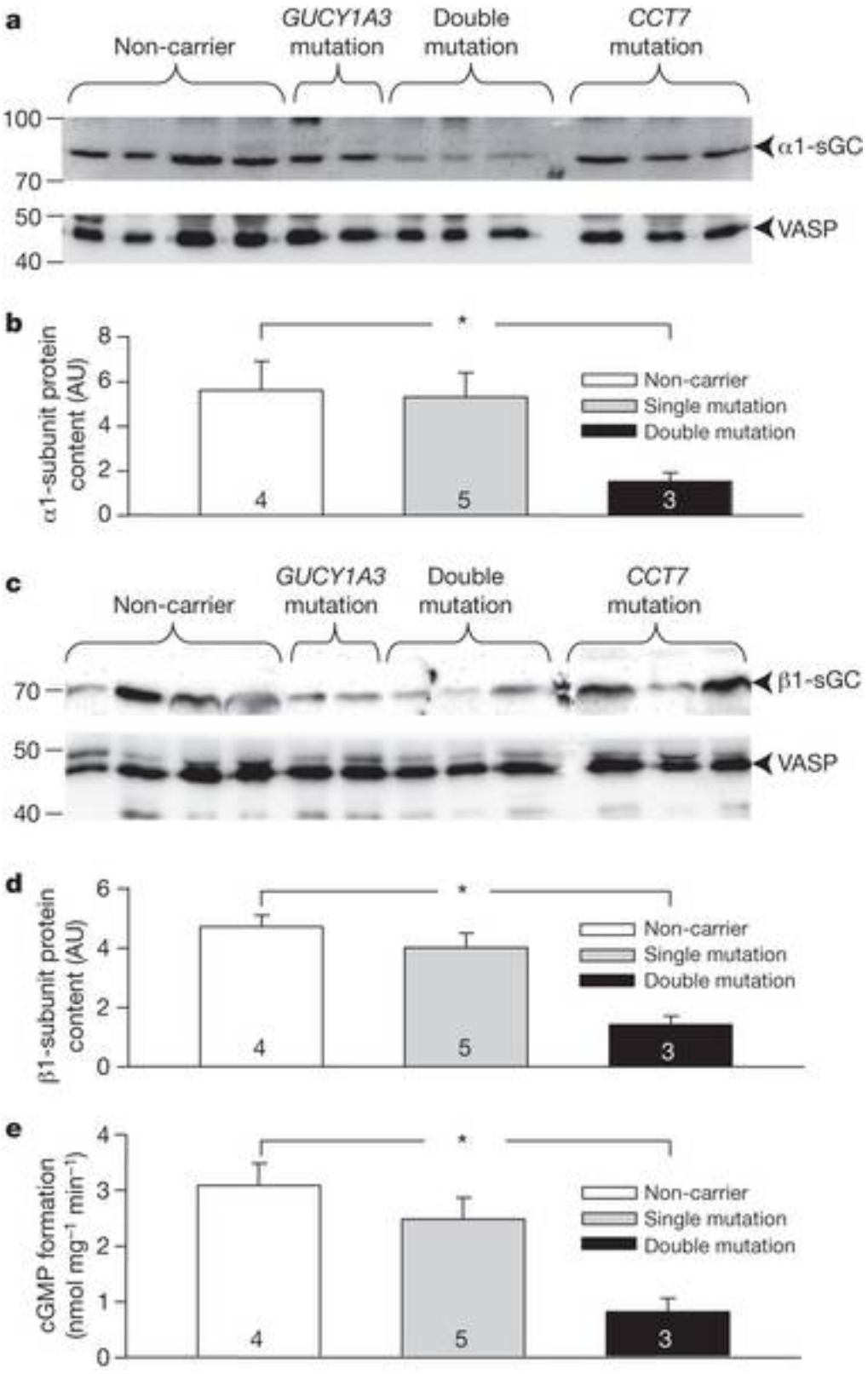


Figure 2: Schematic view of a column diagram and pattern band formed in GUCY1A3 gene in mutant and normal genes.

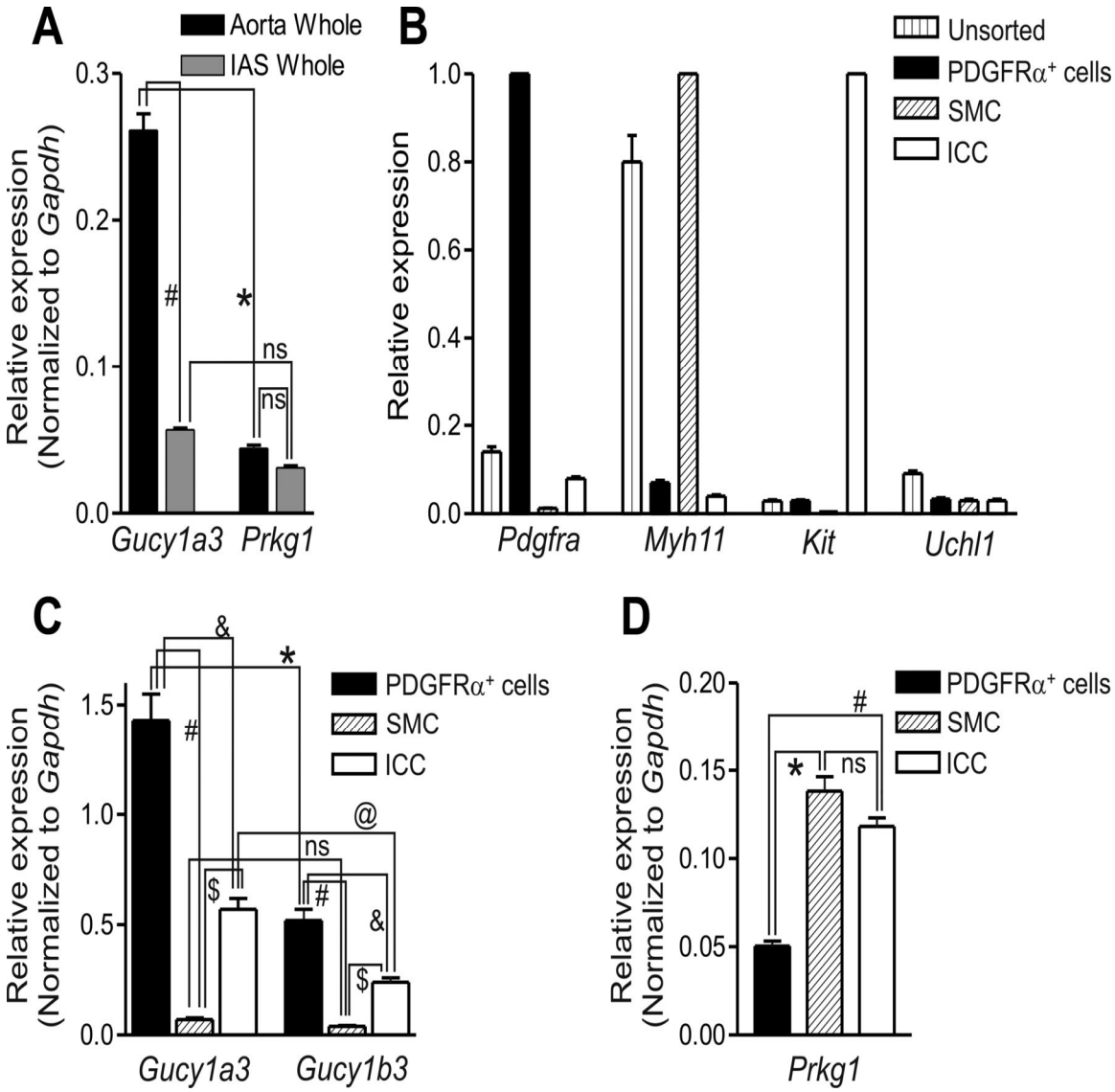


Figure 3: Schematic view of a column diagram of the expression of Genes GUCY1A3 and GUCY1B3 and PRKG1.

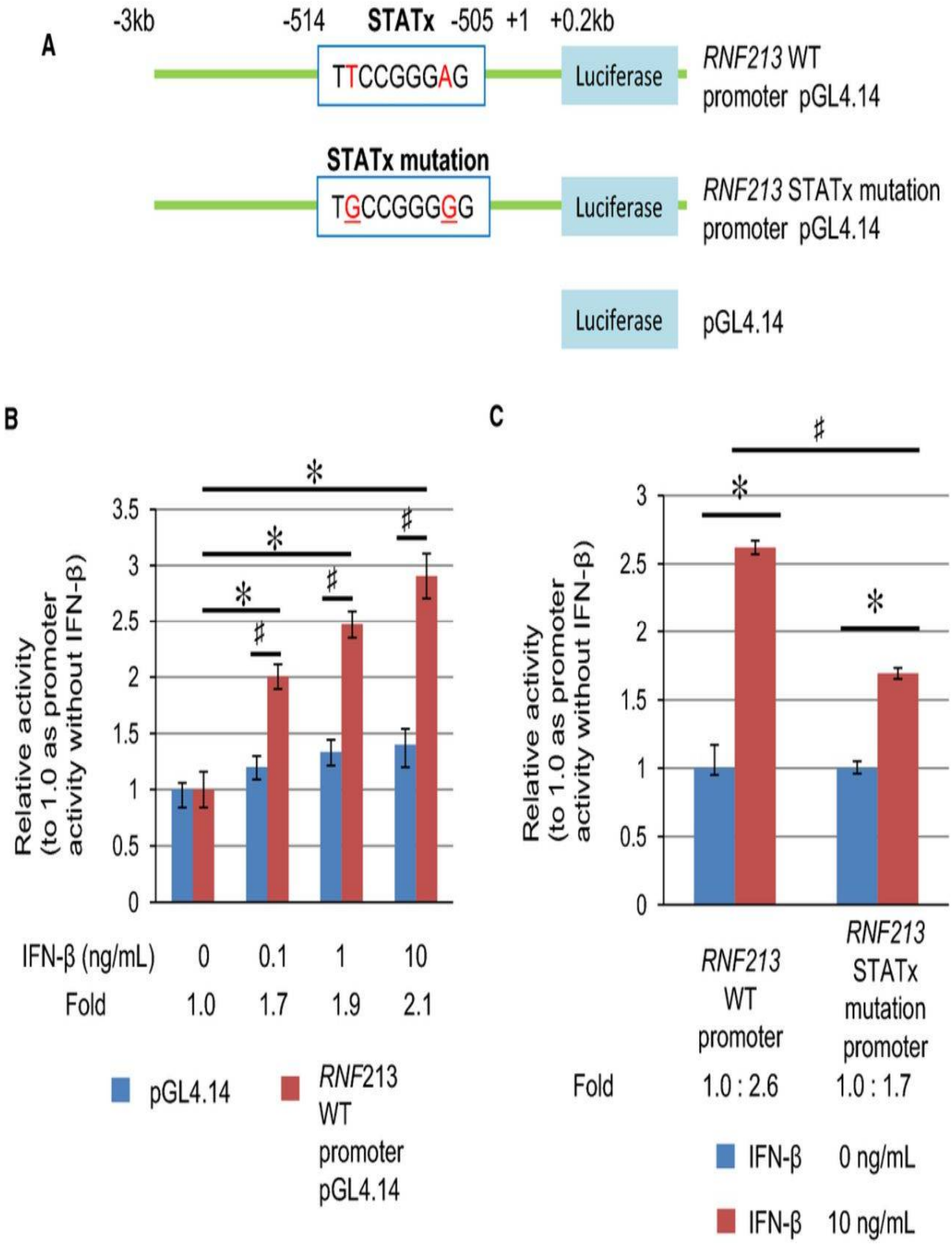


Figure 4: Schematic view of a column diagram RNF213 gene expression compared with normal and mutant genes.

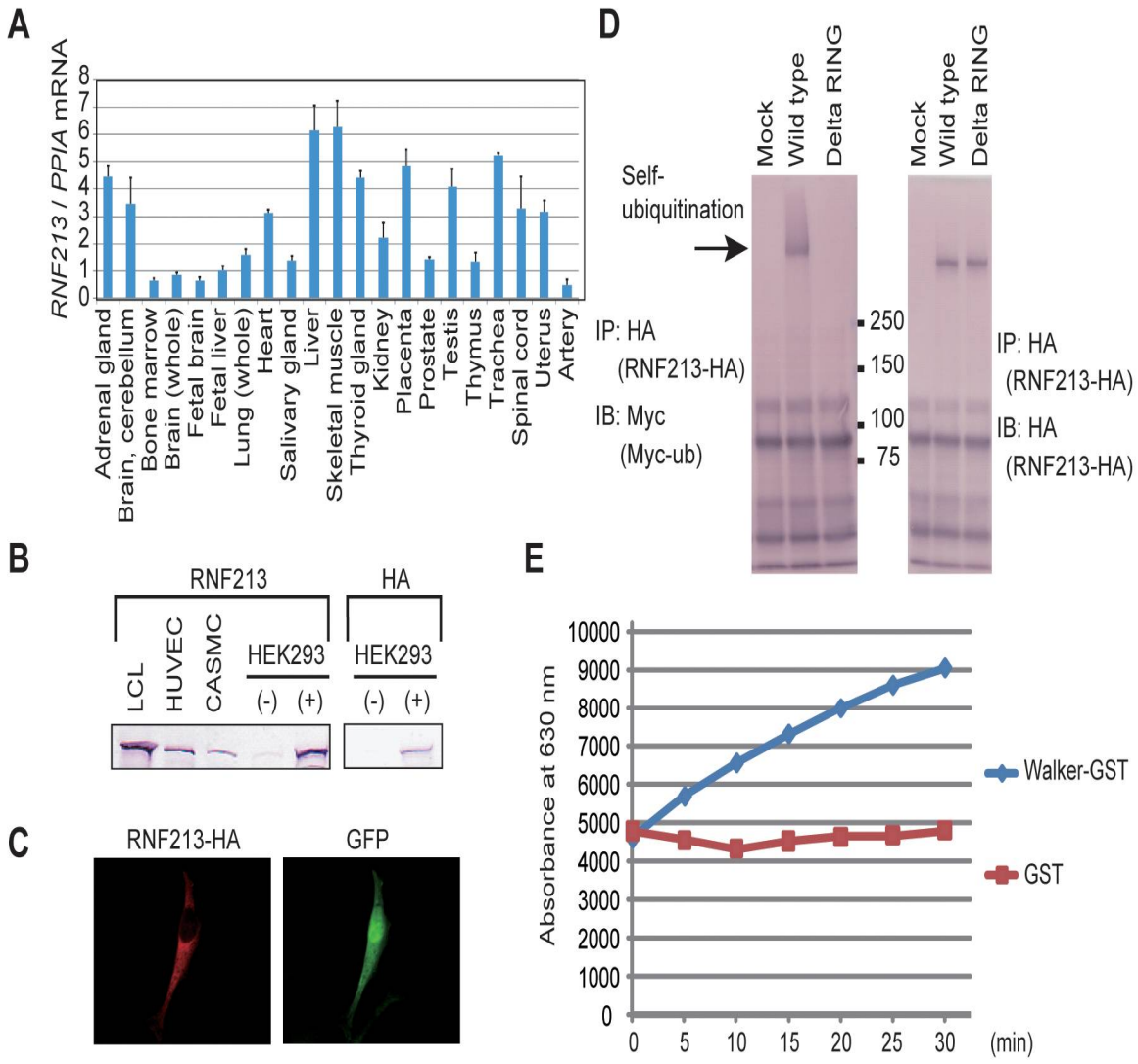
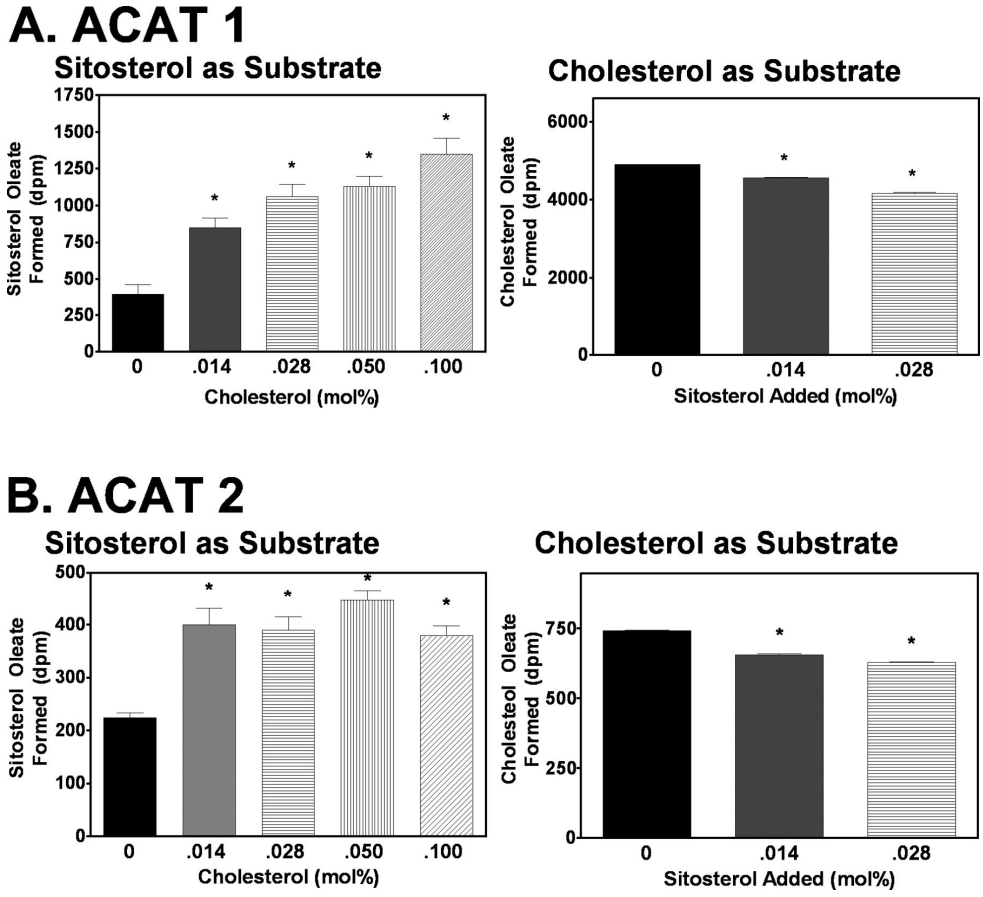


Figure 5: Schematic view of a column diagram and pattern band formed in RNF213 gene in mutant and normal genes.



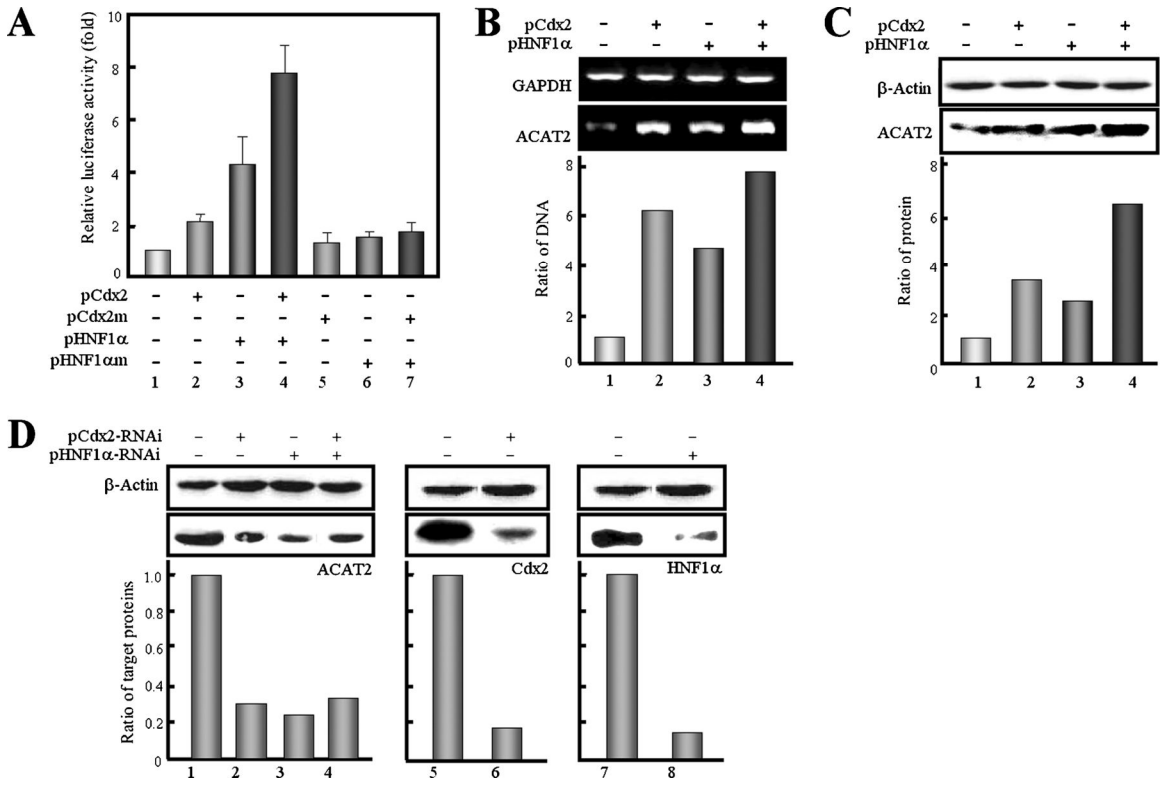


Figure 6: Schematic view of a column diagram and pattern band formed in ACAT2 gene in mutant and normal genes.

Discussion and conclusion

According to the results of sequencing the genome of patients with Moyamoya disease, and the genetic mutations RNF213,ACTA2,GUCY1A3 genes found that about 96% of patients with Moyamoya disease, they have this genetic mutations. Patients with Moyamoya disease, unusual and frightening images in the process of Moyamoya disease, experience. Lot epigenetic factors involved in Moyamoya disease. But the most prominent factor to induce Moyamoya disease, mutations is RNF213,ACTA2,GUCY1A3 genes.This gene can induce the birth and can also be induced in the adulthood.

References

1. Moyamoya Disease and Moyamoya Syndrome, R. Michael Scott and Edward R. Smith, New England Journal of Medicine, 360:1226-1237, March 19, 2009.

2. Wakai K, Tamakoshi A, Ikezaki K, et al. (1997). "Epidemiological features of moyamoya disease in Japan: findings from a nationwide survey". *Clin Neurol Neurosurg*. 99 Suppl 2: S1–5.

3. Hallemeier C, Rich K, Brubb R, Chicoine M, Moran C, Cross D, Zipfel G, Dacey R, Derdeyn CP (2006). "Epidemiological features of moyamoya disease in Japan: findings from a nationwide survey". *Stroke*. **37** (6): 1490–1496.

4. Janda, Paul; Bellew, Jonathan; Veerappan, Venkatachalam (2009). "Moyamoya disease: case report and literature review". The Journal of the American Osteopathic Association. **109** (10): 547–553.

5. Kuriyama S, Kusaka Y, Fujimura M, et al. (2008). "Prevalence and clinicoepidemiological features of moyamoya disease in Japan: findings from a nationwide epidemiological survey". *Stroke. **39** (1): 42–7.*

6. Ma J, Liu Y, Ma L, Huang S, Li H, You C (2013) RNF213 polymorphism and Moyamoya disease: A systematic review and meta-analysis. Neurol India 61(1):35-9.

7. Killory BD, Gonzalez LF, Wait SD, Ponce FA, Albuquerque FC, Spetzler RF (Jun 2008). "Simultaneous unilateral moyamoya disease and ipsilateral dural arteriovenous fistula: case report". *Neurosurgery* Jun;62(6):E1375-6; discussion E1376. **62** (6): E1375–6; discussion E1376.

8. Gilmore, B; Michael, M (2011-02-01). "Treatment of acute migraine headache". American family physician. **83** (3): 271–80.

9. Diener, HC; Charles, A; Goadsby, PJ; Holle, D (October 2015). "New therapeutic approaches for the prevention and treatment of migraine.". The Lancet. Neurology. **14** (10): 1010–22.

10. Armstrong, C; American Academy of, Neurology; American Headache, Society (15 April 2013). "AAN/AHS update recommendations for migraine prevention in adults.". American family physician. **87** (8): 584–5.

11. Vos, T; Flaxman, AD; Naghavi, M; Lozano, R; Michaud, C; Ezzati, M; Shibuya, K; Salomon, JA; et al. (Dec 15, 2012). "Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010". Lancet. **380** (9859): 2163–96.

12. Bigal, ME; Lipton, RB (June 2008). "The prognosis of migraine". Current Opinion in Neurology. **21** (3): 301–8.

13. Buzzi, MG; Cologno, D; Formisano, R; Rossi, P (Oct–Dec 2005). "Prodromes and the early phase of the migraine attack: therapeutic relevance". Functional neurology. **20** (4): 179–83.

14. Rossi, P; Ambrosini, A; Buzzi, MG (Oct–Dec 2005). "Prodromes and predictors of migraine attack". Functional neurology. **20** (4): 185–91.

15. Samuels, Allan H. Ropper, Martin A. (2009). Adams and Victor's principles of neurology (9 ed.). New York: McGraw-Hill Medical. pp. Chapter 10.

16. Tintinalli, Judith E. (2010). Emergency Medicine: A Comprehensive Study Guide (Emergency Medicine (Tintinalli)). New York: McGraw-Hill Companies. pp. 1116–1117.

17. Kaniecki, RG (June 2009). "Basilar-type migraine". Current pain and headache reports. **13** (3): 217–20.

18. Curr Opin Neurol. 2016 Jun;29(3):299-301. doi: 10.1097/WCO.0000000000000310. The migraine postdrome. Bose P1, Goadsby PJ.

19. de Vries, B; Frants, RR; Ferrari, MD; van den Maagdenberg, AM (July 2009). "Molecular genetics of migraine". Human Genetics. **126** (1): 115–32.

20. Montagna, P (September 2008). "Migraine genetics". Expert Review of Neurotherapeutics. **8** (9): 1321–30.

21. Levy D, Strassman AM, Burstein R (June 2009). "A critical view on the role of migraine triggers in the genesis of migraine pain". Headache. **49** (6): 953–7.

22. Martin PR (June 2010). "Behavioral management of migraine headache triggers: learning to cope with triggers". Curr Pain Headache Rep. **14** (3): 221–7.

23. Pavlovic, JM; Buse, DC; Sollars, CM; Haut, S; Lipton, RB (2014). "Trigger factors and premonitory features of migraine attacks: summary of studies.". Headache. **54** (10): 1670–9.

24. Morren, JA; Galvez-Jimenez, N (December 2010). "Where is dihydroergotamine mesylate in the changing landscape of migraine therapy?". Expert opinion on pharmacotherapy. **11** (18): 3085–93.

25. Kelley, NE; Tepper, DE (January 2012). "Rescue therapy for acute migraine, part 1: triptans, dihydroergotamine, and magnesium". Headache. **52** (1): 114–28.

26. Sumamo Schellenberg E, Dryden DM, Pasichnyk D, Ha C, Vandermeer B, Friedman BW, Colman I, Rowe BH (2012). "Acute migraine treatment in emergency settings".

27. Tepper Stewart J., S. J.; Tepper, Deborah E. (April 2010). "Breaking the cycle of medication overuse headache". Cleveland Clinic Journal of Medicine. **77** (4): 236–42.

28. Law, S; Derry, S; Moore, RA (21 October 2013). "Sumatriptan plus naproxen for acute migraine attacks in adults.". The Cochrane database of systematic reviews. **10**: CD008541.

29. Eken, C (March 2015). "Critical reappraisal of intravenous metoclopramide in migraine attack: a systematic review and meta-analysis.". The American journal of emergency medicine. **33** (3): 331–7.

30. Derry S, Moore RA, McQuay HJ (2010). Moore M, ed. "Paracetamol (acetaminophen) with or without an antiemetic for acute migraine headaches in adults". Cochrane Database Syst Rev. **11** (11): CD008040.

Medical laboratory News-ISSN;2345-3427