Journal of Alzheimer's Parkinsonism & Dementia

Research Article

Assessment of Genes Mutations HLA-DQB1, HLA-DR2, HLA-DR14, in Patients with Sleep Disorders and Insomnia Family

This article was published in the following Scient Open Access Journal:
Journal of Alzheimer's Parkinsonism & Dementia
Received July 28, 2017; **Accepted** August 07, 2017; **Published** August 14, 2017

Shahin Asadi*, Mahsa Jamali, Manoush Tohidirad, Hamideh Mohammadzadeh

Young Researchers and Elite Club, Tabriz, Branch, Islamic Azad University, Tabriz, Iran

Abstract

In this study we have analyzed 300 people. 140 patients and 160 control group had sleep disturbances. The genes HLA-DR2 and HLA-DR14 and HLA-DQB1 analyzed in terms of genetic mutations made. In this study, people who have genetic mutations were targeted, with nervous disorders, sleep or slept badly. In fact, of all people with insomnia and bad sleep, 140 people had a genetic mutation in the gene HLA-DQB1. And 6 had a genetic mutation in HLA-DR14 gene were a genetic mutation in the gene HLA-DR2. Any genetic mutations in the target genes control group, did not show.

Keywords: Genetic study, Sleep disorders, Mutation HLA-DR2, HLA-DR14, HLA-DQB1

Introduction

Fatal familial insomnia (FFI) is an extremely rare autosomal dominant inherited prion disease of the brain. It is almost always caused by a mutation to the protein PrPC, but can also develop spontaneously in patients with a non-inherited mutation variant called sporadic fatal insomnia (sFI). FFI has no known cure and involves progressively worsening insomnia, which leads to hallucinations, delirium, and confusional states like that of dementia [1]. The average survival span for patients diagnosed with FFI after the onset of symptoms is 18 months [2].

The mutated protein, called PrP_{Sc}, has been found in just 40 families worldwide, affecting about 100 people; if only one parent has the gene, the offspring have a 50% risk of inheriting it and developing the disease. With onset usually around middle age, it is essential that a potential patient be tested if they wish to avoid passing FFI on to their children. The first recorded case was an Italian man, who died in Venice in 1765 [3].

Materials and Methods

In this study, 140 patients with sleep disturbances and 160 healthy controls 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 [4]. To isolate Neuroglial cells erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solution in ratio of 1 to 5 with 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 sediment with PBS (Phosphate Buffered Saline), pipetazh and slowly soluble carbohydrate ratio of 1 to 2 on ficole (Ficol) was pouredin the 480G was centrifuged for 34 minutes. Mono nuclear Neuroglial cells also are included, has a lower density than ficole and soon which they are based. The remaining erythrocytes has a molecular weight greater than ficole and deposited in test tubes [5].

The supernatant, which contained the mononuclear cells was removed, and the 400 Gera was centrifuged for 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 HLA-DQB1, HLA-DR2, HLA-DR14 and were kept [6].

To determine the purity of Neuroglial cells are extracted, flow cytometry was used.

***Corresponding Author:** Shahin Asadi-Molecular Genetics-Iran-Tabriz, Director of the Division of Medical Genetics and Molecular Research, Iran, Tel: +989379923364, Email: shahin.asadi1985@gmail.com

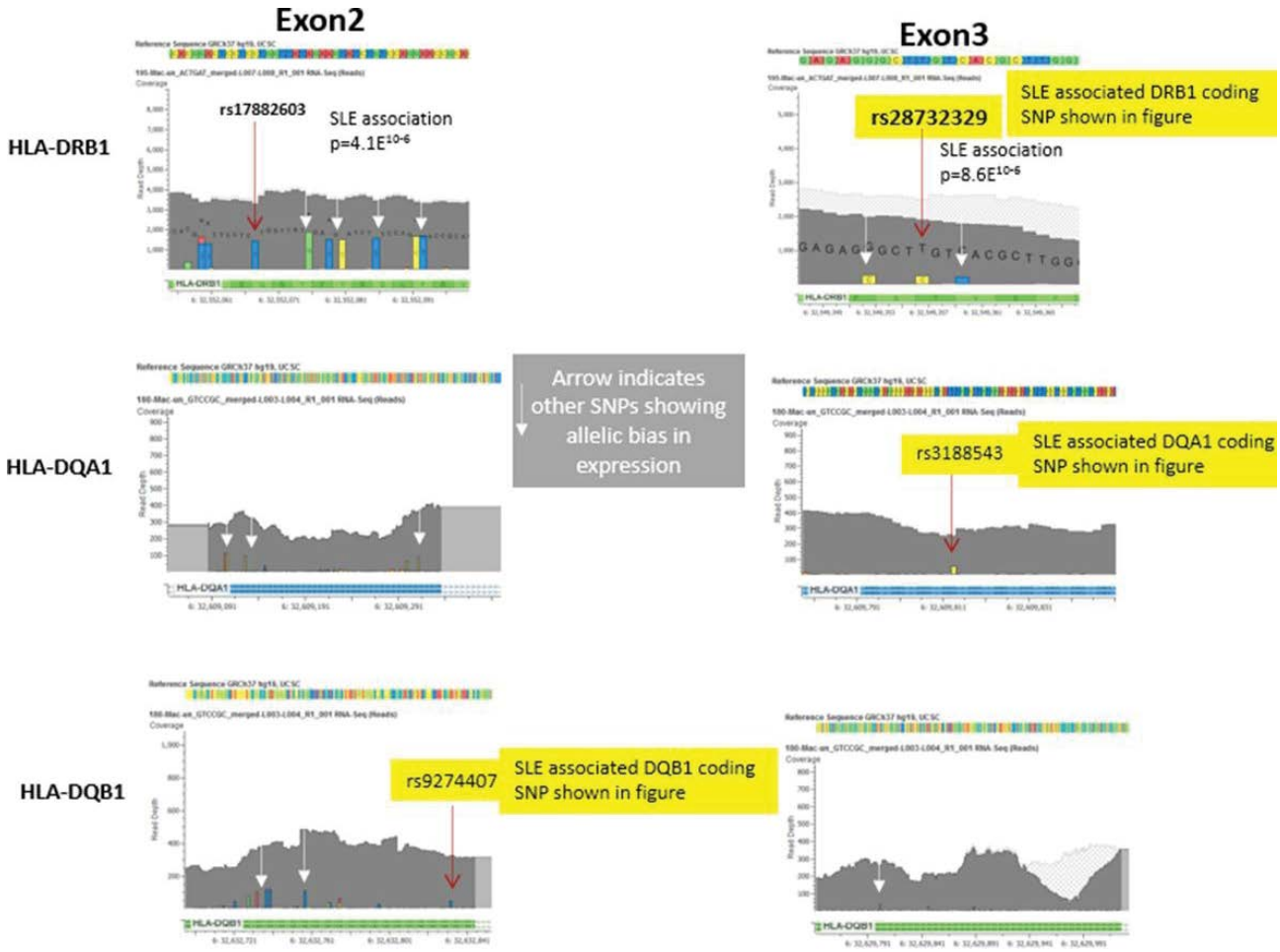

Figure 1: Schematic view of exon HLA-DRB1, HLA-DQA1, HLA-DQB1 polymorphisms in patients with sleep disorders.

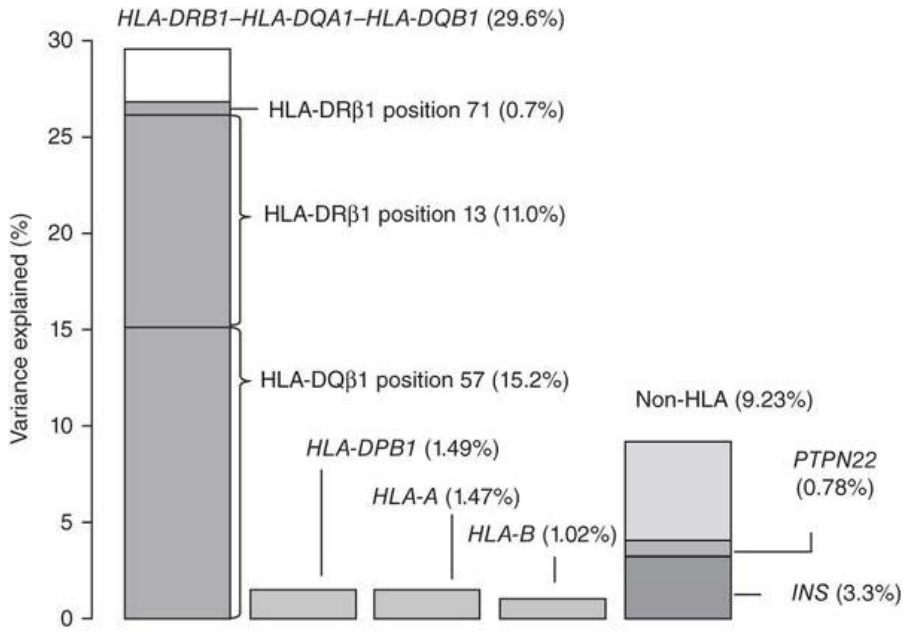

Figure 2: Schematic View of the HLA-DRB1, HLA-DQA1, HLA-DQB1 Variants Diagrams in Patients with Sleep Disorders.

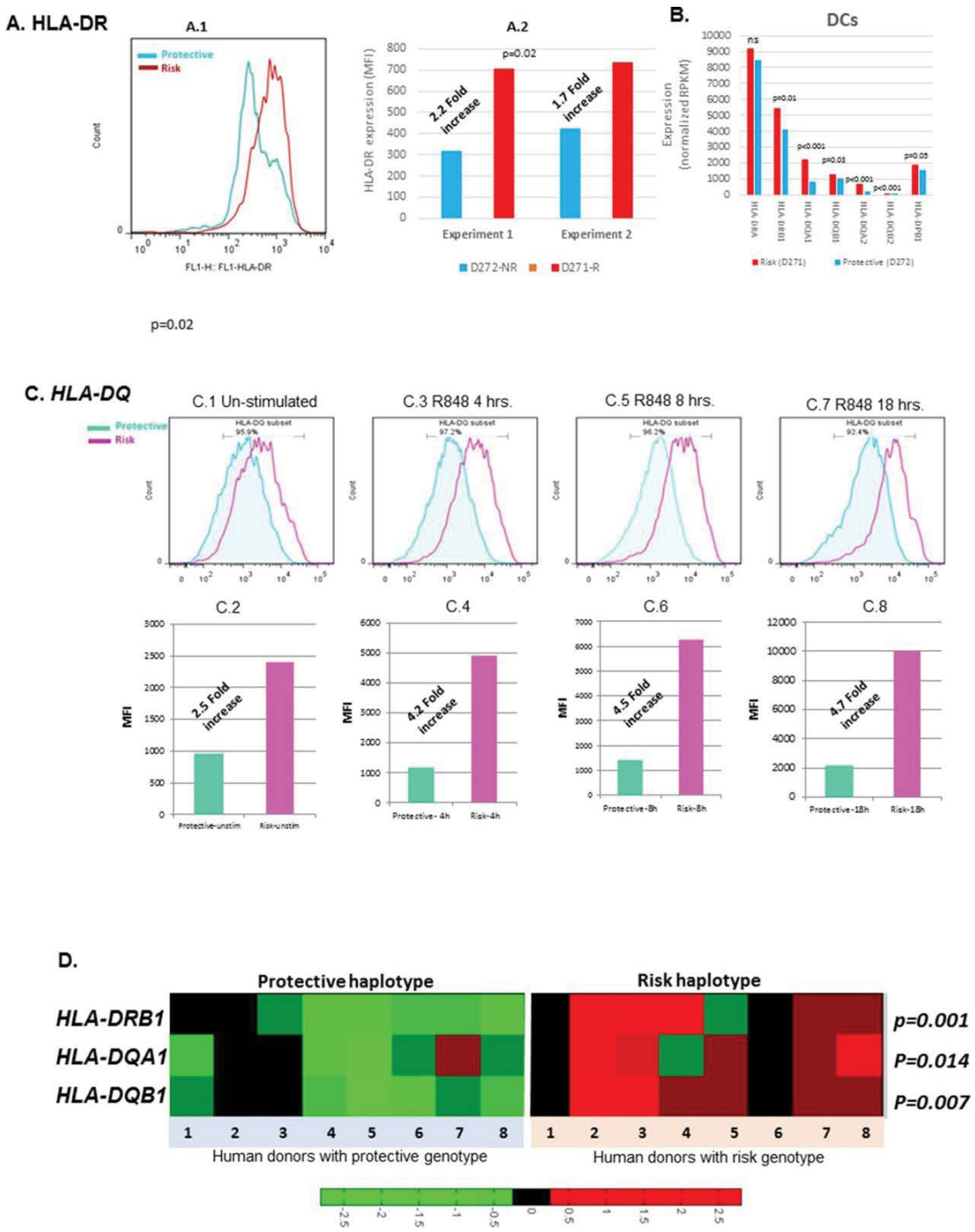

Figure 3: Schematic View of HLA-DRB1, HLA-DQA1, HLA-DQB1 Haplotypes in Patients with Sleep Disorder.

For this purpose, approximately 4-5 × 10^3 Neuroglial cells were transfer red to 1.5 ml Eppendorf tube and then was centrifuged at 2000 rpm for 7minutes a time. Remove the supernatant culture medium and there maining sediment, 100 µl of PBS buffer was added. After adding 5-10 µl $CD4^+$ PE monoclonal anti body to the cell suspension for 60 min at 4°C, incubated and read immediately by flow cytometry. For example, rather than control anti body Neuroglial cells PE, IgG1 negative control solution was used [7].

Total RNA extraction procedure includes:

1. 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200 µl 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 transferred to a new micro tube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hours at -20°C were incubated [8].

2. Then for 45 min at 4°C an was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1 ml 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 20 µl sterile water and at a later stage, the concentration of extracted RNA was determined [9].

To assessment the quality of mi-RNAs, the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentas K1622) and1µl oligoprimers18 (dT) was performed. Following the PCR reaction 2 µM dNTP, 1µg cDNA, Fermentas PCR buffer 1X, 0 / 75µM $MgCl_2$, 1.25 U / µL Tag DNA at 95°C for 4min, 95°C for 30s, annealing temperature 58°C for 30s, and 72°C for 30 seconds, 35 cycles were performed. Then 1.5% agarose gel, the PCR product was dumped in wells after electrophores is with ethidium bromide staining and color were evaluated [10-12] (Figures 1 and 2).

Discussion and Conclusion

According to the results of sequencing the genome of patients with sleep disturbances, and the genetic mutations HLA-DR2, HLA-DR14, HLA-DQB1 genes found that about 100% of patients with sleep disturbances, they have this genetic mutation. Patients with sleep disturbances, unusual and frightening images in the process of sleep disturbances, experience. Lot epigenetic factors involved in sleep disturbances. But the most prominent factor to induce sleep disturbances, mutations is HLA-DR2, HLA-DR14, HLA-DQB1 genes. This genes can induce the birth and can also be induced in childhood and adulthood (Figure 3).

References

1. Chang BS, Lowenstein DH. Epilepsy. N. Engl. J. Med. 2003;349(13):1257-1266.
2. Fisher Robert S, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical clinical definition of epilepsy. *Epilepsia.* 2014;55(4):475-482.
3. Longo, Dan L 2012. 369 Seizures and Epilepsy. Harrison's principles of internal medicine (18th ed.). *McGraw-Hill.* p. 3258.
4. Pandolfo M. Genetics of epilepsy. Semin Neurol. 2011;31(5):506-518.
5. Eadie, MJ. Shortcomings in the current treatment of epilepsy. *Expert Rev of Neurother.* 2012; 12 (12):14191427.
6. Bergey, GK. Neurostimulation in the treatment of epilepsy. *Exp Neurol.* 2013;244: 87-95.
7. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Global Burden of Disease Study 2013, Collaborators. *Lancet.* 386 (9995):743-800.
8. Brodie MJ, Elder AT, Kwan P. Epilepsy in later life. *Lancet Neurol.* 2009;8:(11): 1019-1030.
9. Holmes, Thomas R. Browne, Gregory L. Handbook of epilepsy (4th ed.). Philadelphia: Lippincott Williams & Wilkins. 2008;7.
10. Newton CR, Garcia HH. Epilepsy in poor regions of the world. *Lancet.* 2012;380(9848):1193-2001.
11. Wilden JA, Cohen-Gadol AA. Evaluation of first nonfebrile seizures. *Am Fam Physician.* 2012;86(4):334-340.
12. Berg AT. Risk of recurrence after a first unprovoked seizure. *Epilepsia.* 2008; 49 Suppl 1: 13-18.