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Research Article

Assessment of Genetics Mutation PRNP Gene induction Prion Disease, in Tabriz, Iran

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Abstract

In this study we have analyzed 20 people. 10 patients Prion disease and 10 persons control group. The gene PRNP, analyzed in terms of genetic mutations made. In this study, people who have genetic mutation were targeted, with nervous disorders, Prion disease. In fact, all people with Prion disease. 10 patients Prion disease had a genetic mutation in the gene PRNP Prion disease. Any genetic mutation in the target gene control group did not show.

Keywords: Genetic Study; Mutation; Prion Disease; the Gene PRNP.

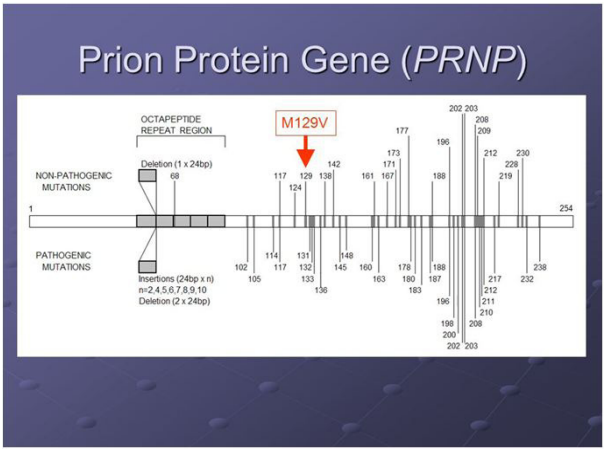

Figure 1: Schematic View of the Molecular Structure of the Prion Protein Gene PRNP with the M129V Mutation.

Introduction

Today, neurological diseases are one of the most important factors in the death. Including the deadly nerve disorders, neurological diseases can be noted Prion disease. This disease induction mutation genetics in gene PRNP. The disease is caused by mutations in genes in nerve cells. But epigenetic factors also have an important role in the ability to induce the disease.

A prion is an infectious agent composed entirely of protein material, called PrP (short for prion protein), that can fold in multiple, structurally distinct ways, at least one of which is transmissible to other prion proteins, leading to disease that is similar to viral infection. They are suspected to be the cause of Transmissible Spongiform Encephalopathy's (TSEs) among other diseases.

Prions were initially identified as the causative agent in animal TSEs such as Bovine Spongiform Encephalopathy (BSE)-known popularly as "mad cow disease"-and scrapie in sheep. Human prion diseases include Creutzfeldt–Jakob Disease (CJD) and its variant (vCJD), Gerstmann–Sträussler–Scheinker syndrome, fatal familial insomnia, and kuru [1]. A 2015 study concluded that Multiple System Atrophy (MSA), a rare human neurodegenerative disease, is caused by a misfolded version of a protein called alpha-synuclein, and is therefore also classifiable as a prion disease [2]. Several yeast proteins have been identified as having prionogenic properties as well [3,4].

A protein as a standalone infectious agent stands in contrast to all other known infectious agents such as viruses, bacteria, fungi, and parasites, all of which contain nucleic acids (DNA, RNA, or both). For this reason, a minority of researchers still consider the prion/TSE hypothesis unproven [5]. All known prion diseases in mammals affect the structure of the brain or other neural tissue; all are currently untreatable and universally fatal [6].

Prions may propagate by transmitting their misfolded protein state: When a prion enters a healthy organism, it induces existing, properly folded proteins to convert into the misfolded prion

form. In this way, the prion acts as a template to guide the misfolding of more proteins into prion form. In yeast, this refolding is assisted by chaperone proteins such as Hsp104p. These refolded prions can then go on to convert more proteins themselves, leading to a chain reaction resulting in large amounts of the prion form [4]. All known prions induce the formation of an amyloid fold, in which the protein polymerises into an aggregate consisting of tightly packed beta sheets. Amyloid aggregates are fibrils, growing at their ends, and replicate when breakage causes two growing ends to become four growing ends. The incubation period of prion diseases is determined by the exponential growth rate associated with prion replication, which is a balance between the linear growth and the breakage of aggregates [7]. The propagation of the prion depends on the presence of normally folded protein in which the prion can induce misfolding; animals that do not express the normal form of the prion protein can neither develop nor transmit the disease.

Prion aggregates are extremely stable and accumulate in infected tissue, causing tissue damage and cell death [8]. This structural stability means that prions are resistant to denaturation by chemical and physical agents, making disposal and containment of these particles difficult. Prion structure varies slightly between species, but nonetheless prion replication is subject to occasional epimutation and natural selection just like other forms of replication [9].

Materials and Methods

In this study, 10 patients with Prion disease, and 10 persons control group were studied. A peripheral blood sample from patients and parents with written permission control was prepared. After separation of serum, using Real Time-PCR technique of tRNA molecules was collected. To isolate Neuroglial cells erythrocytes were precipitated from Hydroxyl Ethyl Starch (HES) was used. At this stage, HES solution in ratio of 1to7with the peripheral blood of patients and controls were mixed [10]. After 60 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 18 min at 600 Gera. The cell sediment with PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrate ratio of 1to4 on ficole (Ficol) was poured in the 780G was centrifuged for 41 minutes. Mono nuclear Neuroglial cells also are included, has a lower density than ficole and soon which they are based. The remaining erythrocytes have a molecular weight greater than fico leand deposited in test tubes [11].

The supernatant, which contained the mono nuclear cells, was removed, and the 600 Gera was centrifuged for 17 minutes. Finally, the sediment cell, the antibody and Neuroglial cells was added after 41 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 gene PRNP, and were kept.

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose, approximately 3-7 × 103Neuroglial cells were transfer red to1.5ml Eppendorf tube and then were centrifuged at 2000 rpm for 9 minutes at atime. 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 4C incubated and read immediately by flow cytometry. For example, rather than control anti body Neuroglial cells PE, IgG1 negative control solution was used [12].

Total mRNA Extraction Procedure includes:

1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200µl chloroform solutions to target mix and then transfer the micro tubes were added, and the shaker well was mixed for 20 seconds. The present mix for 4 minutes at room temperature and then incubated for 20 min at 4Can was centrifuged at 13200 rpm era. Remove the upper phase product was transfer reductase new micro tube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hoursat -20C were incubated [13].

Then for 45min at 4Can was centrifuged at 12000 rpm. 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 [14].

To assessment the quality of mi-RNAs, the Real Time-PCR technique was used. The cDNA synthesis inreverse Transcription Reaction (RT) kit (Ferment as K1931) and 1µl oligoprimers 18 (dT) was performed. Following the PCR reaction 2µM dNTP, 1µg cDNA, Ferment as PCR buffer 1X, 0 / 75µM MgCl2, 1.25 U / µL Tag DNA at 95°C for 4min, 95°C for 30s, annealing temperature 58°C for 30s, and72 °C for30 seconds, 35 cycles were performed. Then 1.5% agarosegel, the PCR product was dumped in wells after electrophores is with ethidium bromide staining and color were evaluated [15].

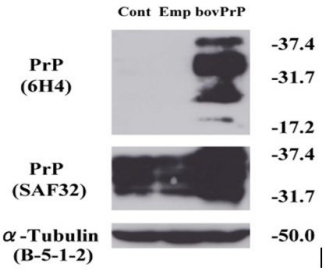

Figure 2: Schematic view of the PRNP gene and alpha-tubulin band pattern formed in the prion protein.

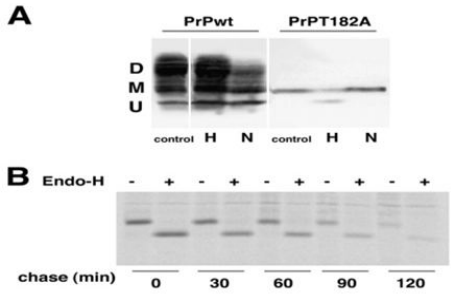

Figure 3: Schematic View of the Pattern Formed in the Band PRNP Gene with Wild Control Group and Patients in the Prion Protein.

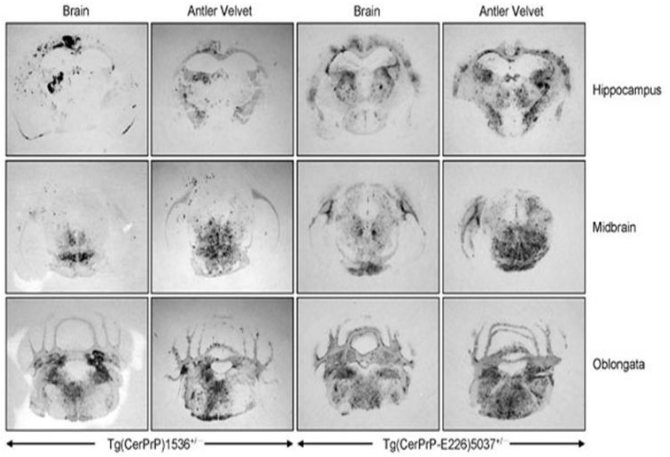

Figure 4: Schematic View of the PRNP Gene in the Brain and the Hippocampus Structural Change in the Wild Control Group and Patients in the Prion Protein.

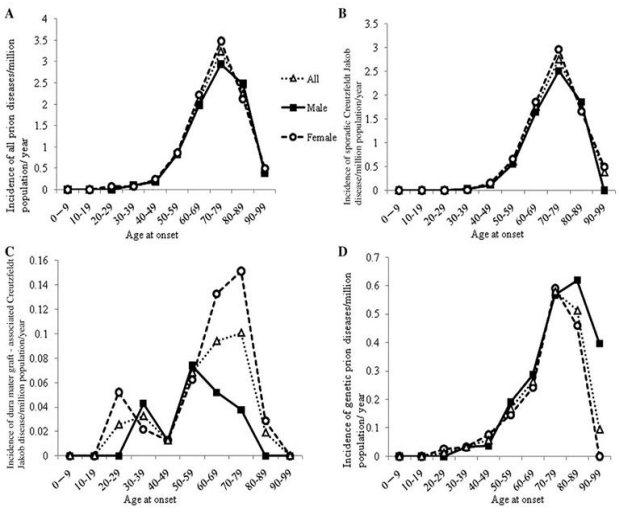

Figure 5: Schematic View of Statistical Diagrams in Patients with Jacob and Prion Disease the Genders At Different Ages Of Life.

Discussion And Conclusion

According to the results of sequencing the genome of patients with Prion disease, and the genetic mutation PRNP gene found that about 100% of patients with Prion disease, they have this genetic mutation. Patients with Prion disease, unusual and frightening images in the process of Prion disease, experience. Lot epigenetic factors involved in Prion disease. But the most prominent factor to induce Prion disease, mutation is PRNP gene. This gene can induce the birth and can also be induced in the adulthood.

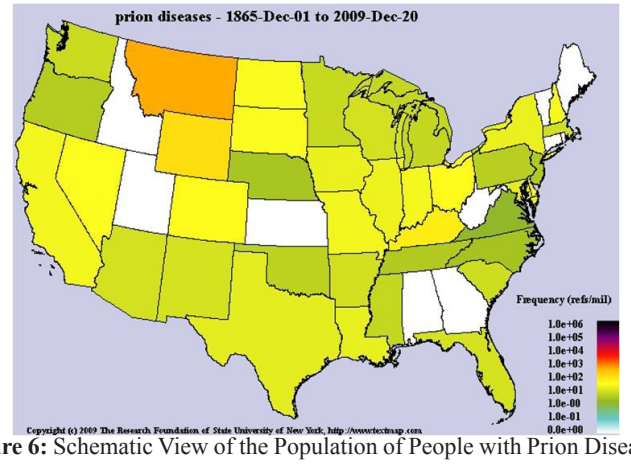

Figure 6: Schematic View of the Population of People with Prion Disease around the World from December 1, 1865 to December 20, 2009.

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