# Damage of Brain Wiring in Amyotrophic Lateral Sclerosis

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On a show on Discovery channel telecasted in May 2010, person was sitting in a wheelchair attached to a computer to assist him to speak in arobotic voice "Hello, My name is Stephen Hawking, physicist, cosmologist and something of a dreamer. Although I cannot move and have to speak through a computer, in my mind I am free."Because of my keen interest in biology, this voice intrigued me to know about the disease he had more than the 'big bang theory' he discovered. I learnt that at the age of 21, he was diagnosed with the disease known as "Amyotrophic Lateral Sclerosis" (ALS) or "Lou Gehrig's Disease". In our childhood, we have seen people who are paralyzed, and who have lost the sensation in some parts of their body. On reading, I realized that ALS is a condition that involves loss of control over motor function. A person with ALS can sense the outer stimuli but fail to respond to it. The cells, known as neurons or more specifically motor neurons that process the signal from brain to the muscle and other body parts, become weak and degenerate with time and lead to loss of motor functions.

*My tribute to Prof. Stephen Hawking for bravely surviving ALS (photo atMadame Tussauds, 2018)*

* Mr. Abhishek Vats, Ph.D. Scholar from Sir Ganga Ram Hospital, New Delhi, is pursuing his research on "Role of Protein Homeostasis Pathways in Amyotrophic Lateral Sclerosis." His popular science story entitled "Damage of Brain Wiring in Amyotrophic Lateral Sclerosis" has been selected for AWSAR Award.

This can be explained by taking a simple example, movement of an electrical fan. If we consider a case where a mouse cuts the wire connected to a fan, no matter how hard you press the switch, the fan will not function. The fan is equivalent to our muscle or body part, the on/off switch is our brain and the electric wire is the motor neurons through which the impulse travels. If this wiring is damaged (degenerated) as in ALS, the brain is not able to send the signal to the muscle and leads to loss of muscle movement as illustrated below in the right panel. Unfortunately, this drastically affects the daily life activities in ALS patients and eventually makes them dependent on family members/ caregivers. If limbs are affected, the patient becomes wheelchair-bound, need assistance for activities including holding a spoon, pen and buttoning a shirt. If bulbar muscles are involved, patients find it difficult to speak or swallow food. Most of the ALS patients die within three to five years and very few live over 10 years with the life support system like ventilator for breathing support. After knowing about ALS, I felt that the disease of the nervous system is probably more frustrating and devastating than deadly cancer. This motivated me to pursue research in the field of neurodegeneration.

I got an opportunity to work on ALS during my Ph.D and focusedon what causes disconnection between the brain and muscles or other body parts. Undiagnosed or suspected ALS patients usually come to the neurology clinic with their family members with doubts, anxiety and fear but in hope as well. In my study, I proposed to evaluate the blood cells of ALS patients with the expectation to see if examining these cells can tell us about this brain disorder. I sawsome changes in blood cells which reflect the changes in brain cells of ALS patients. My work took another direction when a brother and sister visited the neurology clinic at Sir Ganga Ram Hospital. While noting the family history, we found out that the father and an elder sister had died due to similar conditions. It appeared to be a rare familial form of ALS. I decided to study the family to see if any genetic change is associated with the disease in the family. I requested them to participate in the study and they gladly agreed for the betterment of science. I analyzed the genes which have been shown in other populations to be responsible for causing ALS. Surprisingly, no one had examined these genes in the Indian population so far. I found a new genetic change in SOD1 gene in both the siblings which might be the root -cause of their condition. A genetic change is similar to a presence of a red pearl in a white pearl necklace as shown in upper left panel of the illustration. The necklace loses its regularity and appearance. The genetic changes involve abnormal changes in the sequence of the nucleotides (i.e. ATGC) in the DNA. Here, in these siblings the nucleotide guanine (G) replaced by thymine (T) alters the sequence of nucleotide codon from TTG (decoded into Leucine (L) amino acid) to TTT (decode into Phenylalanine (F) amino acid) at 84$^{th}$ position in the SOD1 protein (L84F SOD1). Bioinformatics analysis suggested that this genetic change might be the causal factor of the disease. I, further, wanted to see the penetrance of the genetic change in the family. Without disclosing the genetic change, the neurologist approached the patients and described my interest to study the whole family.

The affected brother who was already on a wheel -chair was highly supportive and gathered his family. I collected the blood samples of 11 members of the family and did the same genetic analysis. Unfortunately, two of the family members: the 23- year- old son of the male patient and

the 28- year-old daughter of the deceased female were having the same genetic mutation. These members did not show any symptoms of ALS. One of the younger sisters of the siblings was not available to give the consent at that time and recently visited the neurology clinic with similar symptoms. After genetic analysis, I found that she and her 11- year- old son also carried the same genetic change. This took a huge emotional toll on me but I took it as a challenge to see how this genetic change in one gene can cause such a devastating disease. One interesting aspect I realized while comparing the clinical features of the three affected siblings. Despite the same mutation, there was difference in the age of onset, the site where symptoms appeared first, severity of disease and survival time after the onset of the disease. All this suggested that ALS is a complex disorder and involves interplay of genetic and additional factors. Though identification of mutation solved one part of the riddle and can be equated to the mouse who chews the electric wire of the fan but what are the molecular consequences of the mutation still remained a mystery. This is a huge concern for the younger asymptomatic members who have this causal mutation and may develop ALS.

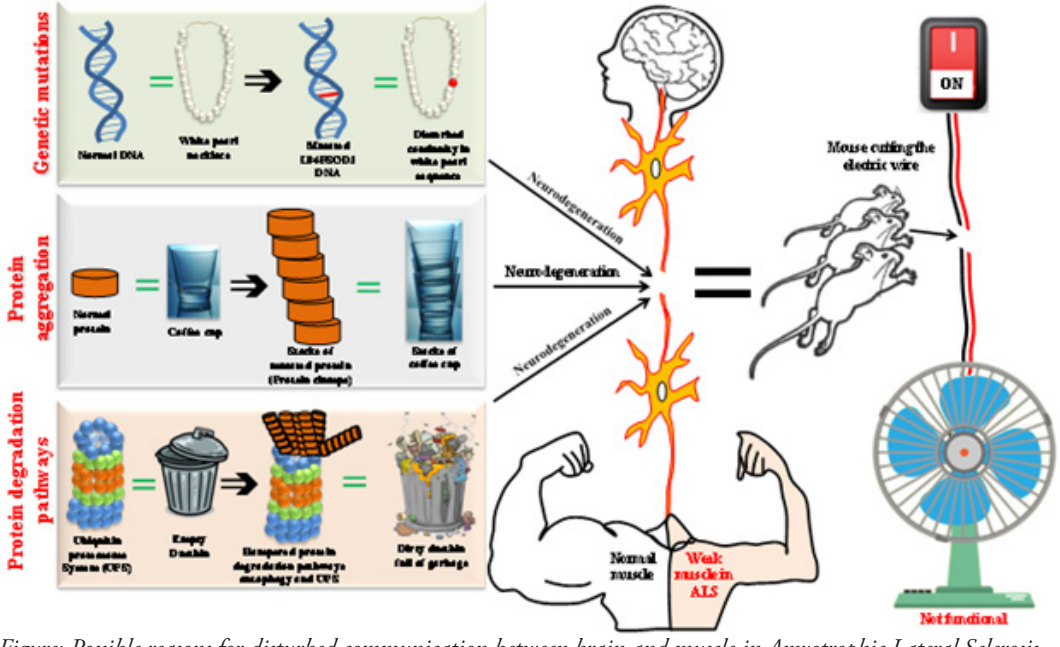


*Figure: Possible reasons for disturbed communication between brain and muscle in Amyotrophic Lateral Sclerosis.*

As it is difficult to examine the motor neurons from the patients, I decided to develop a motor neuron cell model to study the effect of the SOD1 mutation present in these patients. I cloned and expressed the mutation in motor neuronal cells and examined them. Inside the cell, two subunits of normal SOD1 (wild type) join to carry out the function. Interestingly, I observed that SOD1 mutation destabilizes the protein and it does not form the functional dimer. Moreover, this mutant SOD1 protein becomes sticky and forms clumps (aggregates) in the motor neurons as

illustrated below in middle left panel. Even clinically, similar protein clumps have been observed in brains of people who died of ALS. Hence, these clumps appeared to be the culprit. These protein clumps are equivalent to the trash/ garbage of the cell and needs to be removed. At our homes, absence or inefficiency of the garbage truck/ person leads to heap of trash which results in unhygienic and unhealthy condition. As shown in lower left panel of the illustration, cells have well-defined mechanism for garbage disposal and if this mechanism is hampered, the unwanted or aged stuff from the cells is not removed. This accumulation of unwanted material in the cell can lead to diseased condition. The mutant SOD1 clumps are also not cleared by the cell and cells undergo degeneration. I found that these cells are under oxidative stress. I, further, examined the protein degradation machinery (garbage disposal system) which has two components: autophagy and ubiquitin proteasome system. Interestingly, modulating the protein degradation machinery had an effect on mutant SOD1 clumps. This machinery can now be further studied to see if it can be exploited to develop effective therapies for this devastating disorder in the future.

To summarize, I have been able to place the three pieces of the puzzle: genetic mutation, protein aggregates and protein degradation pathways. However, ALS is a complex disorder and requires many more pieces to be joined to explain the disease. This is the reason why it is difficult to predict the outcome of a mutation in members who do not show any symptoms. This makes the counselling a challenge for the clinicians and genetic counsellors.