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Research Article

Finding a possible biomarker to tackle Parkinson's disease by splice analysis and random point mutations to counter the expression of genes involved in Parkinson's disease

Piyusha Kalwad[1], Ranjitha Guttapadu[1] and Pradeep S[1*]

[1]Department of Biotechnology, BMS College of Engineering, Bangalore, India

Abstract

The genes showing aberrant alternative splicing in Parkinson's disease namely SNCA, SNCAIP, LRRK2, SRRM2, MAPT and PARK2 were analysed. Two of the genes, namely SNCAIP and SRRM2 that showed high effect were taken and splice site analysis was carried out. Random mutations were carried out on these two genes using Human Splicing Finder tool and the mutations showing the most promising results (i.e., mutations that can restore natural gene expression) were appropriately chosen to tackle Parkinson's disease.

Keywords: Parkinson's disease; phylogenetic analysis; splice site analysis; mutations, Human Splicing Finder; biomarker discovery

Introduction

There are many diseases which are known to affect the nervous system in humans. One such disease is Parkinson's disease. It is a disorder caused due to aberrant alternative splicing events. (Fu *et al*., 2013).

The alternative splicing process involves removal of the intronic regions from the RNA primary transcript and simultaneous assembly of the exonic regions in different combinations to form a mature mRNA, which then undergoes post transcriptional modification, and gets translated into protein. (La Cognata *et al*., 2015).

Parkinson's disease (PD) mainly affects the motor neuron system. The most common symptoms which are observed are shaking, slowness in movement, difficulty in walking etc. Other symptoms also include sensory, sleep and emotional problems. Several studies show the involvement of alternative splicing in nervous system diseases. Alternative splicing events in the nervous system play an important role in ion transportation, neurotransportation, memory, and learning. (Fu *et al*., 2013).

There are six genes which are known to be involved in aberrant alternative splicing in Parkinson's disease. Two of

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[1]***Corresponding author**
Pradeep S,
Department of Biotechnology, BMS College of Engineering, Bangalore, India
Email: padees@gmail.com

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these genes, SRRM2 and SNCAIP (codes for synphilin-1), show the most promising effect.

Eyal et al. (2006) and Humbert *et al*. (2007) have shown that synphilin-1A (lacking exons 3 and 4 and containing exon 9A) is present in PD. Overexpression of synphilin-1A causes proteasome saturation; is aggregation enhancing; and directly promotes the inclusion, formation, and neurotoxicity of proteins, which indicates that this isoform may contribute to neuronal degeneration.

Shehadeh *et al*. (2010) performed exon microarray analyses from the peripheral blood of 17 PD patients. They found a noteworthy upregulation of the upstream (5′) exons of SRRM2 and a downregulation of the downstream exons, which caused downregulation of the long isoform. (Fu *et al*., 2013).

Thus, we targeted these genes and tried to counter their regulation to restore original gene expression.

The sequences of these genes were obtained and splice site analysis was performed. Random mutations were carried out in these sequences using online software tools. The effect of these mutations was analysed to check the possibility of upregulation and downregulation.

The main aim of this study was to counter-regulate the gene expression in the alternatively spliced genes.

Tools and Databases

1. The website 'HUMAN SPLICING FINDER' was used to carry out the splice site analysis and mutations. This website allowed us to carry out the splice site analysis by giving the input as the gene name and the specific exon number being targeted.
2. NCBI was used as a resource to find out the sequences of the respective genes. The gene name was entered and the specific sequence was downloaded in FASTA format, both for nucleotide and protein.
3. EBI tools like CLUSTALW were used to carry phylogenetic analysis. CLUSTALW took all the sequences (of all six genes) and generated a phylogram (with evolutionary distances) and cladogram (without evolutionary distances).

Results

SNCAIP

Synphilin-1A (lacking exons 3 and 4 and containing exon 9A) is over expressed in PD. We chose the transcript of Exon 9 containing 11 exons.

Over expression of synphilin-1A causes proteosome saturation. (Fu *et al*., 2013) Thus, mutations were carried out on Exon 9 of SNCAIP gene to decrease the expression of exon 9. Various mutations were carried out (insertions, deletions, substitutions, indels and duplications) with an aim to find silencer motifs which could silence the expression of SNCAIP. However, upon analysis in Human Splicing Finder, we could not obtain any silencer motifs. Although we couldn't find silencer motifs, the presence of breakage of the potential splice sites and enhancer motifs indicates that there would be a negative expression and these sites would no longer be a part of the exon. This would make the site unrecognisable for alternate splicing, or would decrease the possibility of alternate splicing. Additionally, the enhancer motifs also showed a negative variation indicating a decrease in the expression of the exon (Fig. 1 to 4).

Fig. 1: Potential splice sites. The picture above shows the potential splice sites of exon 9 of SNCAIP before carrying out mutations. This gives probable locations where the splicing could occur.

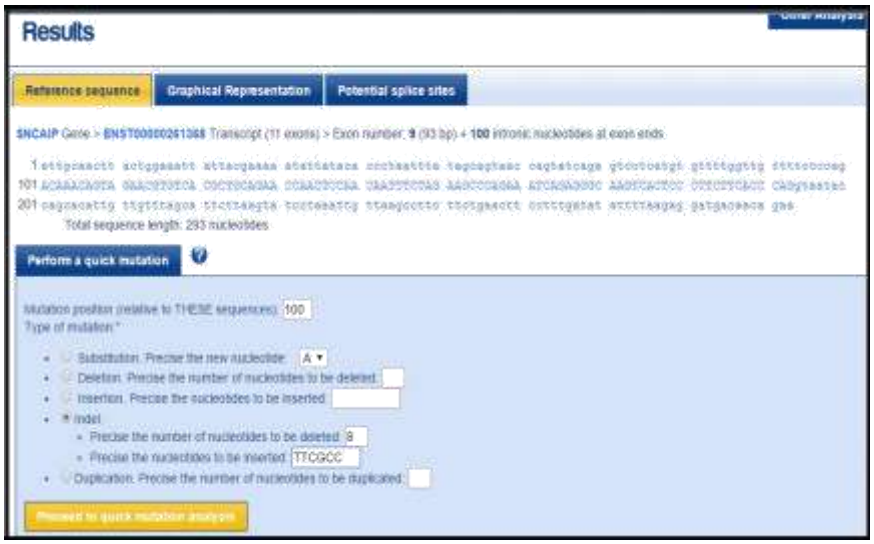

Fig. 2: The picture above shows an INDEL: Insertion of the nucleotide sequence 'ttcgcc' and deletion of 8 nucleotides at position 100. Many different combinations of indels, duplications and substitutions were carried out to study the effect of mutations. The mutation showing the best result (as we could not find any silencer motif) was the above mutation.

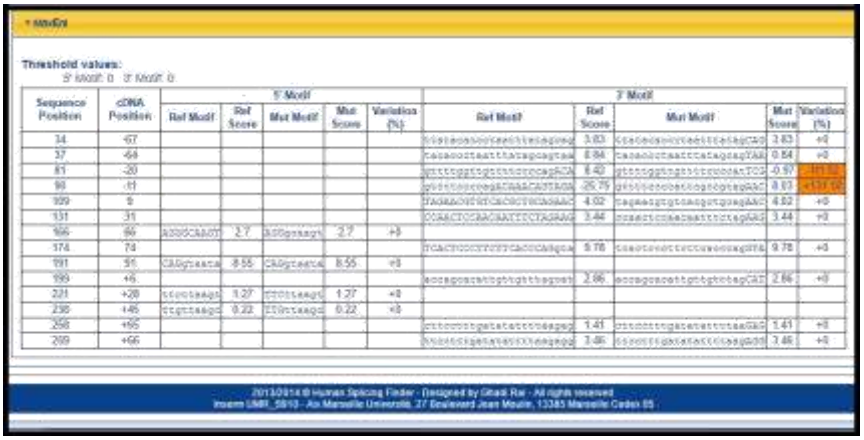

Fig 3: The figure above shows the Maximum entropy values after splicing. Entropy values indicate the stability of the sequence after mutation. The variation in the entropy values after splicing has been shown in the labelled rows.

Fig. 4: The figure above shows the potential splice sites after the above-mentioned mutation was carried out. 3 sites were broken and 1 new site was formed

The best result that we obtained after performing mutations showed 3 potential splice sites broken and 5 enhancer motifs sites broken. As seen above, the location of the broken potential splice sites can be easily observed. In the following picture, the broken enhancer motif sites can be observed.

Along with this, three new sites are also formed.

The breakage of the potential splice sites and the enhancer motifs indicate the loss of function of those motifs which enhance the gene regulation. Thus, the gene now is downregulated.

This is the desired level of SNCAIP gene under normal condition as opposed to the overexpressed level observed in Parkinson's disease.

SRRM2

Exon 1 – One of the upstream exons which is upregulated in PD. Transcript containing 15 exons was chosen (Fig. 5 to 11).

Random mutations were carried out to find silencer motifs which would bind to these upregulated sequences and silence their expression. But we were unable to find such silencer motifs. So, in the place of silencer motifs we went on to find if any sites in the potential splice sites were broken. Once the sites are broken, they would not be recognised and hence the alternative splicing at these sites would either reduce or be completely prevented.

On carrying out many random mutations, we found that on the insertion of 4 nucleotides (ATTT) at position 2, 4 potential splice sites were broken. This could be a possible strategy to reduce the alternative splicing of Exon1.

Insertion of 'attt' was carried out at position 2:

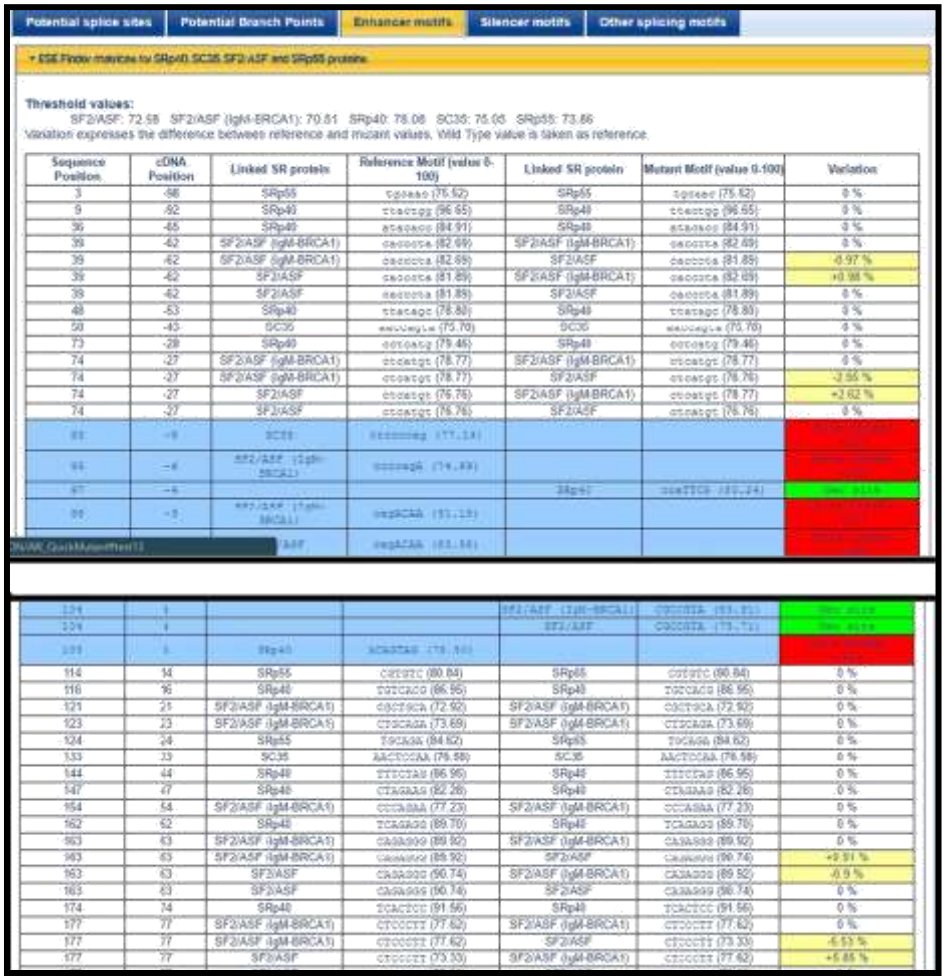

Fig 5: The figure shows the breakage of sites in Enhancer motifs that indicates the loss of function of the enhancer motifs that help in the up-regulation of the gene. Thus, the gene is successfully downregulated.

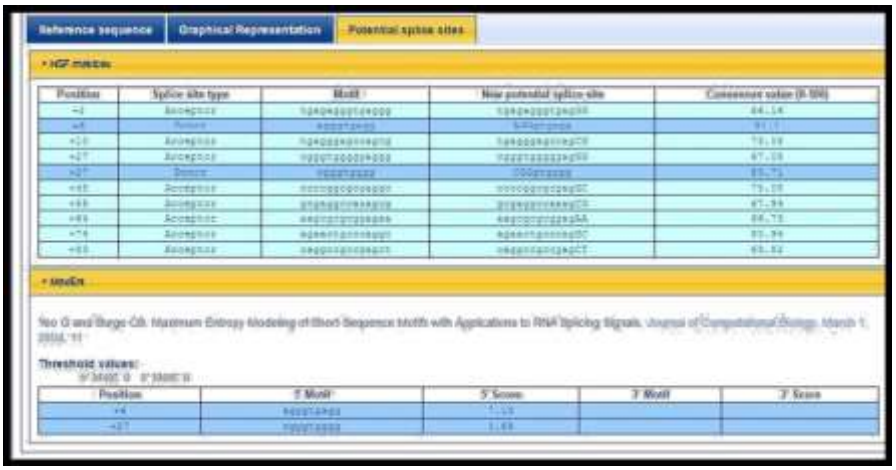

Fig. 6: The figure above shows the potential splice sites in the exon sequence before mutating.

Fig. 7: The figure above shows the Potential splice sites after the mutation was carried out. Three potential splice sites were broken.

The effect of mutation is seen to have brought the breakage of 3 potential splice sites, thus reducing the probability of alternative splicing occurring in those regions.

Exon 15- One of the downstream exons that is down regulated in PD. Transcript containing 15 exons was chosen.

Since this is one of the exons in the gene that is down regulated during Parkinson's disease, we tried to find enhancer motifs which would cause the up-regulation of the exon. From many random mutations, it was found that many point mutations led to the formation of enhancer motifs.

Some of the sites in these enhancer motifs were broken, while some had new sites formed. This caused a variation in the expression. One of the mutations, which was a duplication of 8 nucleotides at the 8th position gave the maximum positive variation and hence was chosen as the most efficient strategy for the up-regulation of exon15, SRRM2 gene expression.

Duplication of 8 nucleotides at position 8

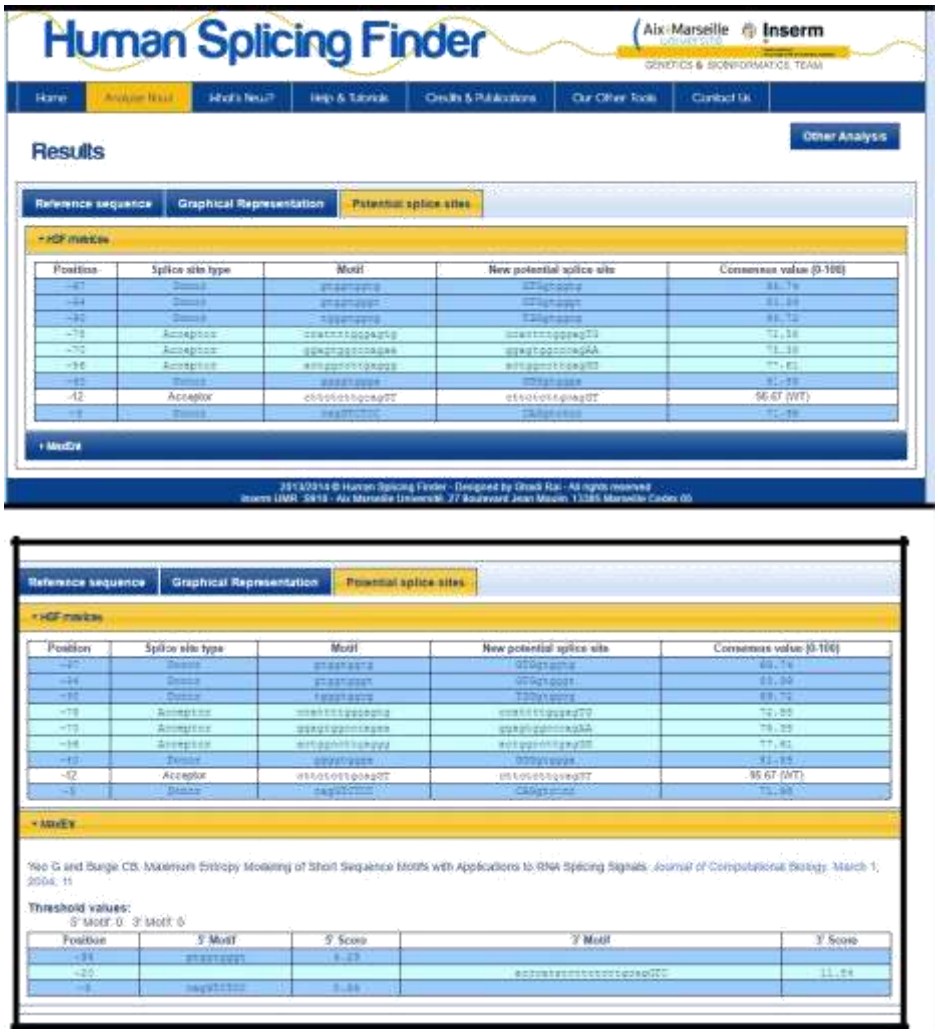

Fig. 8: This figure above shows the Potential splice sites and entropy modelling that represents the stability of the gene sequence before carrying out mutations.

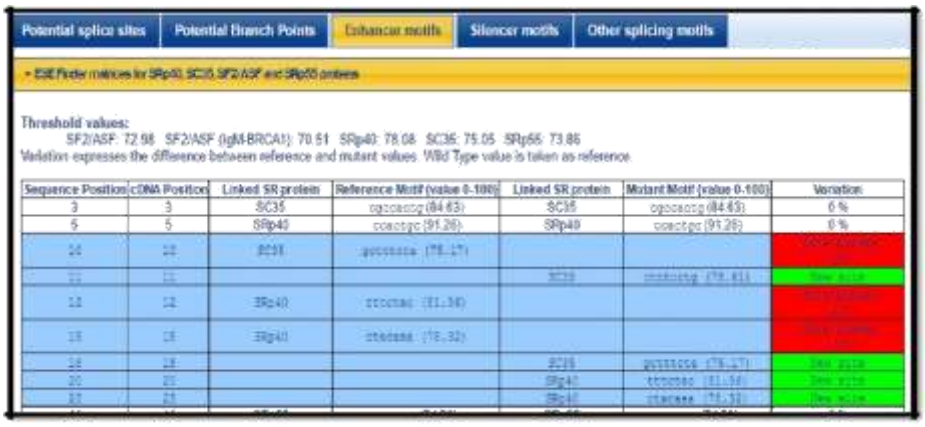

Fig. 9: The figure above shows the Enhancer motifs after the above-mentioned mutation. Four new sites were formed while three sites were broken. The enhancer motifs variation represents the difference between the reference and the mutant values. The wild type value was taken as reference.

Fig. 10: The above figure shows the potential splice sites after the mutation. Two sites are observed to be broken while two new sites are formed. The broken splice sites reduce the possibility of alternative splicing occurring at that region.

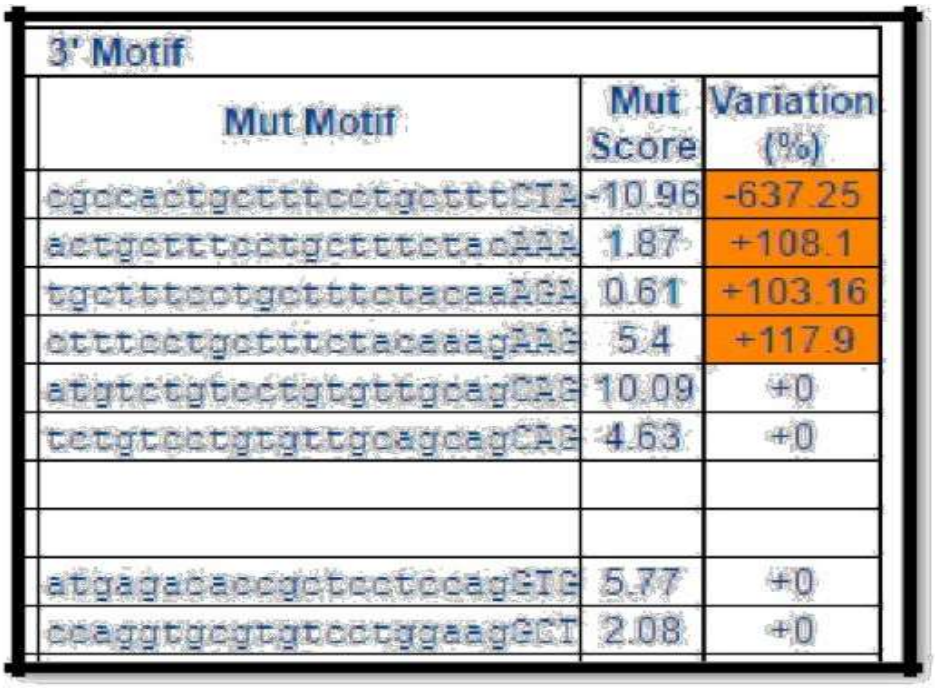

Fig. 11: The figure above shows the Entropy variation after mutating. A large positive variation is observed on an average indicating good stability of the sequence after mutating.

Table 1: Consolidated Results

Gene	**Regulation To Restore Original Gene Functionality**	**Potential Splice Sites**	**Enhancer Motifs**	**Result**
Sncaip (Exon 9)	Downregulation	Broken- 3 Formed – 1	Broken – 5 Formed - 3 (Negative Variation)	Down bregulated
Srrm2 (Exon 1)	Downregulation	Broken – 4	_	Down regulated
Srrm2 (Exon 15)	Upregulation	Broken – 2 Formed – 2	Broken – 3 Formed – 4 (Max Positive Variation)	Up regulated

The Table 1 summarizes our study on these two genes. It depicts the result of random mutations on the expression of these genes.

The future scope of study of this project involves the study of the extent to which the expression of the genes alters to develop an effect therapy to tackle Parkinson's disease. Probable wet lab studies including site directed mutagenesis along with techniques such as FISH can help to validate the results.

Discussion

Alternative splicing is a key element in eukaryotic gene expression that increases the coding capacity of the human genome and an increasing number of examples illustrate that the selection of wrong splice sites cause various human diseases (Tazi *et al*., 2009). Improper selection of splice sites leads to differential gene expression. We thus targeted the differentially expressed genes with an aim to restore their native gene expression.

The expression of genes is controlled by various external and internal factors. Some of them include enhancer motifs and silencer motifs. Enhancer motifs are sequences that direct accurate slicing of heterogeneous nuclear RNA to pre mRNA or mRNA. Thus, their presence leads to up regulation of genes. While silencer motifs are sequences that bind to enhancer motifs and prevent up regulation of genes. Enhancer motifs have been shown to bind negative regulators belonging to the heterogeneous nuclear ribonucleoprotein (hnRNP) family (Sironi *et al*., 2004).

Thus, to restore original gene expression of the alternatively spliced genes (whose differential expression led to Parkinson's disease), we looked for silencer motifs for differentially upregulated genes and enhancer motifs for differentially down regulated genes.

We also tried out random point mutations in the sequences of the alternatively spliced genes to see if we can obtain the enhancer or silencer motifs required. Insertions and deletions were also carried out to see if any of the sites break or new sites is formed as origins of replication could conceivably be made non-functional by mutations that change, delete or disrupt sequences recognized by the relevant binding proteins (Genomes 2, Chapter 13).

Based on the number of sites broken and number of sites formed the regulation of expression can be manipulated. For example, by mutating Exon 9 of SNCAIP we found that 3 potential splice sites are broken. This implies that the original binding sites for enhancer motifs or inducers of splicing are unavailable. Thus, the up regulation of this gene will be disrupted. Similar analysis was carried out for the other genes.

The future scope of study of this project involves the study of the extent to which the expression of the genes alters to develop an effect therapy to tackle Parkinson's disease. Probable wet lab studies including site directed mutagenesis along with techniques such as FISH to validate the results can be carried out.

Conclusion

The expression level of genes plays an important role in various cell signalling pathways in the body. Up-regulation of genes leads to their over expression while down-regulation leads to their under expression. Both the conditions lead to the alteration of homeostasis and may lead to a probable disorder. Two such genes involved in the development of Parkinson's disease were targeted in this study.

Genes that have undergone aberrant alternative splicing leading to the development of the disease were randomly mutated in such a way that the expression of up-regulated genes is decreased and expression of down-regulated genes is increased thus restoring their normal gene expression and indirectly their function.

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