Microsatellite Plots in SOD-1 gene with Mutations and Predicted Domains reveals a possible role of Mutagenesis

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ABSTRACT

Approximately 10% of Amyotrophic lateral sclerosis/ Motor Neuron Disease (ALS/MND) is inherited of which up to 20% is caused by mutations, (changes) in the superoxide dismutase 1 (SOD1) gene. Detailed bioinformatics study of all the known mutations in the SOD1 gene revealed interesting information. The information about all the experimentally proven mutations in the SOD1 gene was collected and analyzed using bioinformatics tools and software programs. We computationally tried to find out whether the presence of microsatellite plot in the SOD1 gene has any significance in the generation of these mutations. Our analysis revealed that we found 13 microsatellites out of which 7 microsatellites regions have found to be mutated and have fallen inside domain region. The mutation falling in the domain region seems to be inducting a change in the secondary structure and resulting in change or absence of protein function. Thus indicating a positive role of microsatellites in mutagenesis.

Key Words: Microsatellites, Mutations, ALS (Amyotrophic lateral sclerosis), MND (Motor Neuron Disease).

INTRODUCTION

ALS is the most common form of motor neurone disease (MND) and involves both upper motor neurones (those running from the brain to the spinal cord) and lower motor neurones (those running from the spinal cord out to the muscles) [1]. About 10% of all ALS cases are familial (FALS), meaning that they are caused by inherited genetic damage known as mutations [2-5]. Inheritance of damage to the enzyme CuZn superoxide dismutase (SOD1) is involved in about 20% of FALS [6-8]. Earlier work has provided evidence that (1) mutations impair the stability of SOD1 molecules so that they fall apart, and (2) that the pieces of the molecule then clump together or stick to other molecules in the neuron, creating obstructions to normal processes [9]. These factors are the most probable reasons for SOD1 toxicity and for disease progression [10-11].

Mice with a mutation in the gene for the transport of protein, dynein, ('LOA mice') develop weakness in midlife and a mild loss of motor neurons [12]. This mild syndrome is due to the mutated dynein, which is one of the main motors of nerve cells, and which transports cargoes through motor neurons back to the cell body, result of this mutation, some LOA mice have slower retrograde transport (transport back to the cell body) than normal mice [13]. Recent research shown that 'LOA/SOD1' mice – which are offspring from a mating of LOA mice to SOD1mice, and which carry both mutations – live longer than their siblings, which have the mutant SOD1 gene alone. Unexpectedly, the presence of dynein mutation is protecting these mice from the effects of SOD1 mutation [14-15].

The cause(s) of MND are largely unknown but the strongest clues have come from the 10% of patients who have a family history of MND [6] [9]. There is also Growing evidence that genes influence susceptibility in anybody who develops MND [17]. Nerve cells contain various structures, all performing different functions. Some of these structures are damaged in MND [11]. Excessive amounts of a chemical called glutamate, found in the central nervous system, cause damage to motorneurones in MND. TNFα is made in the spinal cord and previous Research has shown that it is present at higher levels in people with MND compared to the general population. TNFα makes motorneurones more susceptible to the damaging effects of glutamate by increasing the ability of glutamate to bind to motor neurons at specific receptor sites ('calcium-permeable AMPA receptors') .Mutations in SOD1 gene are known to be involved in several disorders, including Amyotrophic lateral sclerosis(ALS) and Other Motor Neurone Diseases [16].

The human genome also consists of a large number of nucleotide repeat units of size 1-6bp repeated tandem called Microsatellites or Simple Sequence Repeats(SSRs) or Short Tandem Repeats(STRs) [18].Microsatellites are found in all the known genomes, spanning from prokaryotes, eukaryotes and viruses and are widely distributed both in coding and non-coding regions [19-20]. Mutations in these microsatellite regions occur at much higher rate when compared with those in the rest of the genome [21].

Microsatellites are known to be highly polymorphic due to other high rate of mutations in their tracts [22]. These mutations can be either in the form of increase/decrease of repeat units or in the form of single nucleotide substitutions/deletions/insertions and other events [23]. Increase or decrease of repeat units of microsatellites in coding regions might lead to shift in reading frames thereby causing changes in protein product [24] and in non-coding regions are known to effect the gene regulation .Point mutations (Substitutions and Indels) are also found to occur at a higher rate in microsatellites than elsewhere. Microsatellite mutations with in or near certain genes are known to be responsible for some human neurodegenerative disease [25]. So, we made a brief study to check whether the mutations in this SOD1 gene have any relation with these microsatellite plot and the study revealed interesting results.

METHODS and MATERIALS

We have obtained the sequence from the NCBI database and we used the IMEX tool for Extracting the Microsatellite plots [26].In this we used the advanced mode with the default values for the main parameters. All the experimental proved mutations of the SOD1 gene, that are falling inside the coding regions and eventually leading to phenotypic differences were collected from the Human Gene Mutation Database(HGMD) [27]. A DNA to Amino Acid translator is used for converting nucleotide sequence into protein sequence Since microsatellites are drawn from the nucleotide sequence and HGMD mutations are given for protein sequence. we analyzed the HGMD mutations within the microsatellites regions of SOD1 gene have fallen in the functional domains,repeats,motifs, features by using the Simple Modular Architecture Research Tool (SMART)[28] .The results are given in the table1.

The table 1below gives the list of mutations considered for analysis from HGMD.[29-40]

S.No	Codon Change	Amino Acid Change	Codon Number	Phenotype	Reference
1.	gTGC-GGC	Cys-Gly	6	Amyotrophic lateral sclerosis	Kohno (1999) Neurosci Lett **276,** 135
2.	GTG-GAG	Val-Glu	7	Amyotrophic lateral sclerosis	Hirano (1994) Biochem Biophys Res Commun **204,** 572
3.	gCTG-GTG	Leu-Val	8	Amyotrophic lateral	Andersen (2003)

				sclerosis	Amyotroph Lateral Scler Other Motor Neuron Disord **4,** 62
4.	aGGA-AGA	Gly-Arg	37	Amyotrophic lateral sclerosis	Rosen (1993) Nature **362,** 59
5.	aCTG-GTG	Leu-Val	38	Amyotrophic lateral sclerosis	Rosen (1993) Nature **362,** 59
6.	CTG-CGG	Leu-Arg	38	Amyotrophic lateral sclerosis	Boukaftane (1998) Can J Neurol Sci **25,** 192
7.	GCT-GTT	Ala-Val	89	Amyotrophic lateral sclerosis	Rezania (2003) Amyotroph Lateral Scler Other Motor Neuron Disord **4,** 162
8.	tGCT-ACT	Ala-Thr	89	Amyotrophic lateral sclerosis	Andersen (2003) Amyotroph Lateral Scler Other Motor Neuron Disord **4,** 62
9.	GAC-GCC	Asp-Ala	90	Amyotrophic lateral sclerosis	Anderson (1995) Nat Genet **10,** 61
10.	GAC-GTC	Asp-Val	90	Amyotrophic lateral sclerosis	Morita (1998) Eur J Neurol **5** 389
11.	gATC-TTC	Ile-Phe	104	Amyotrophic lateral sclerosis	Ikeda (1995) Neurology **45,** 2038
12.	TCA-TTA	Ser-Leu	105	Amyotrophic lateral sclerosis	Andersen (2003) Amyotroph Lateral Scler Other Motor Neuron Disord **4,** 62
13.	aCTC-GTC	Leu-Val	106	Amyotrophic lateral sclerosis	Rosen (1993) Nature **362,** 59
14.	GGA-GTA	Gly-Val	108	Amyotrophic lateral sclerosis	Orrell (1997) Eur J Neurol **4** 48
15.	ATC-ACC	Ile-Thr	112	Amyotrophic lateral sclerosis	Esteban (1994) Hum Mol Genet **3,** 997
16.	ATCa-ATG	Ile-Met	112	Amyotrophic lateral sclerosis	Garcia-Redondo (2002) Muscle Nerve **26,** 274
17.	ATT-ACT	Ile-Thr	113	Amyotrophic lateral sclerosis	Rosen (1993) Nature **362,** 59
18.	cATT-TTT	Ile-Phe	113	Amyotrophic lateral sclerosis	Andersen (2003) Amyotroph Lateral Scler Other Motor Neuron Disord **4,** 62
19.	gGTG-CTG	Val-Leu	118	Amyotrophic lateral sclerosis	Andersen (2003) Amyotroph Lateral Scler Other Motor Neuron Disord **4,** 62
20.	gGTG-TTG	Val-Leu	118	Amyotrophic lateral sclerosis	Shimizu (2000) Neurology **54,** 1534
21	AGT-AAT	Ser-Asn	134	Amyotrophic lateral sclerosis	Watanabe (1997) Hum Mutat **9,** 69

The above listed codon changes are considered since they are falling in the Microsatellite regions.

Microsatellite Table:

S.No	Microsatellite Region		No. of iterations	Microsat-ellite Tract length	Left Flank Sequence	Microsatellite Sequence	Right Flank Sequence
	Start Position	End Position					
1.	18	25	2	8	CGAAGGCCGT	GTGCGTGC	TGAAGGGC
2.	113	120	2	8	AGCATTAAAG	GACTGACT	GAAGGCCTGC
3.	266	271	2	6	GGCAATGTGA	CTGCTG	ACAAAGATGG
4.	314	323	5	10	GATTCTGTGA	TCTCACTCTC	AGGAGACCAT
5.	336	341	2	6	GAGACCATTG	CATCAT	TGGCCGCACA
6.	353	358	2	6	GGCCGCACAC	TGGTGG	TCCATGAAAA
7.	397	402	2	6	AGGTGGAAAT	GAAGAA	AGTACAAAGA

The above table gives the list of all the microsatellite regions that are having mutations inside that region.

The results obtained from the SMART :

Confidently predicted domains, repeats, motifs and features:

Name	Begin	End	E-Value
Pfam:Sod_Cu	2	154	1.10e-98

Results:

DNA elements composed of short tandem repeats of 1–5 bp. These sequences are particularly prone to frame shift and mis sense mutations by insertion–deletion loop formation during replication. The mismatch repair system is responsible for correcting these replication errors, and mutation rates are significantly elevated in the absence of mismatch repair.[41]and Due to these mutations during PCR, stutter patterns may appear in the final PCR product, which hinder us from accurate genotyping (genitical information)[42] so keeping the above things in mind we analyzed and found that Out of the 21 mutations which are fallen in the regions of the Microsatellites all of them have fallen in the regions of the functional domains of the SOD1 gene .since the functional domains are the main regions responsible for the function of that gene and any mutations in these regions may cause change in the functionality of the gene. In this study we observed that due to continuous mutations in the microsatellite region may cause the entire microsatellite region is changed to non microsatellite regions.It is shown as an example.
The Single Microsatellite Region in the SOD1 gene Sequence starts at [314] and ends at [323].
Left Flanking Sequence: GATTCTGTGA
Microsatellite Sequence: TCTCTCTCTC
Right Flanking Sequence: AGGAGACCAT
301 GAA GAT TCT GTG ATC TCA CTC TCA GGA GAC (Normal)
301 GAA GAT TCT GTG TTC TTA GTC TCA GGA GAC (Mutant)
100 E D S V I S L S G D
(Normal)

100 E D S V P L V S G D
(Mutant)

Figure 1: Alignment of the normal and mutant SOD1 sequence depicting the change in the amino acid sequence. The Yellow colored site is the Microsatellite region in the Normal Amino acid sequence and Green colored site is the continuous mutations occurred in the Microsatellite region of the SOD1 gene.

Conclusion

Microsatellites are known for their higher rate of mutations and are known to be associated with various diseases. The Insilco analysis of the SOD1 gene mutations has revealed a interesting results and it has shown a possible association with the micro satellites. These mutations from HGMD database are mapped on to the micro satellite tracts and the results seem to indicate that micro satellites play an important role in mutagenesis and by mapping the same with the functional domains we can say that these can cause functionality changes of the SOD1 gene. All the mutations that have been mapped on to the microsatellites are in the functional domain region. Hence microsatellites have played a major role in the SOD1 gene.

This work can be extended further on large scale on the entire human genome in order to get a better evidence on the role of microsatellites in generating mutations.

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