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Original Article

Huntington's chorea, a neurological disorder of all ages – Bioinformatics approach for its precise diagnosis

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ABSTRACT

Background: The disease that obstructs social movements of people is Huntington's disease (HD). Hence, the expedition of its molecular aspects and bioinformatics tools relating to precise confirmation of the disease is warranted. Due to social stigma, the care and attention to safeguard these patients had increased at community level.

Objective: The objective of the study was to explore bioinformatics tools to trace the defects in Huntington's gene and design its small-guided RNA (sgRNA).

Methodology: The HTT gene sequence was retrieved, CRISPR sites were identified, and gRNA sequence was determined using the University of California Santa Cruz (UCSC) Genome Browser. HTT protein molecular structure elucidation was retrieved through PDB and Swiss Model. Ramachandran plot displayed the cluster of poly-Q at the Phi (−60–−36) and Psi (−60–−65) regions. The pattern of residues in the plot displayed that the HTT protein is alpha-helical predominant.

Results: The CRISPR sites on HTT gene are viewed and sgRNA sequences are obtained through the UCSC Genome Browser. This sgRNA sequence along with Cas9 would be planned for genome editing in future experimental models. The Ramachandran plot for HTT protein derived through online Rampage revealed the recurrent appearance of polyglutamine (Q) at the Phi (−55–−65) and Psi (120–135) regions.

Conclusion: Online bioinformatic tools such as UCSC Genome Browser, Swiss-Model, and Rampage help in exploring molecular basis of HD and disease-causing protein HTT, and the same invariably assists in creating awareness among health workers.

Keywords: Cytosine, adenine, and guanine repeat, diagnosis, HTT, Huntington's chorea, poly-Q

Introduction

Huntington's chorea is known in the medical dictionary since the year 1842. Thirty years later, George Huntington coined the name for this disease as Huntington's chorea.[1,2] This Huntington's disease (HD) is a neurodegenerative disorder due to autosomal inheritance [Figure 1]. Its inception is normally found between 40 and 50 years of age. It is reported in the year 1983 that the gene for HD represents from chromosome number 4 and in the year 1993, its gene was unravelled. Incidentally, the pre-manifest diagnosis of HD was made involving trinucleotide repeats of cytosine, adenine, and guanine (CAG).[3] Thus, HD was recognized as a typical model for other genome-based diagnostics in medical domain. CAG is a repeated trinucleotide sequence for more than 30 times in HD gene and this triplet codes for an amino acid, glutamine (Q). Numerous symptomatic related treatments are in vogue; still, there is a need to explore for novel suitable drugs. The seminal attributes of HD are cognitive, psychiatric, and motor disorders. The average age at the beginning of HD is usually within the age range of 30 and 50 years. The average prevalence of the disease is for 17–20 years. As the disease progresses, there is more dependency and requires an attendant.

Behavior and psychiatric symptoms and signs of HD

Dementia

The decline of cognitive ability was found to be a predominant sign among the sufferers of HD. However, HD was found often very mild. The cognitive disabilities are importantly pertaining to the discharge/executive functions. In healthy conditions, these behavioral patterns are goal directed as well

as planned. Further, healthy subjects are capable to differentiate between what is applicable and what is to be ignored; however, patients with HD deformities lose these features. In addition, HD patients are incapable to organize their own life activities or to device their calendar which in the previous years were found easy. All psychomotor processes were found brutally decreased.[3]

Secondary consequences

Weight loss was reported among HD patients. As more personal care and attention are now given to these HD patients, the weight loss looks to be meager. However, it is also noticed that no correlation prevails between weight loss and chorea.[4] Importantly, hypothalamic neuronal loss could be a causative factor.[5,6]

Juvenile HD (JHD)

The incipient symptoms of HD begin to appear either before or at the age of 20 years, and then, it is confirmed as JHD.[7] Of the symptoms, behavioral disturbances and learning and consolidation difficulties are the first normally noticed at school. Chorea is rarely seen within the first 10 years of age and found to manifest in the consequent decade. Epileptic fits, changes in coordination, ability to learn, personality, speech, and behavior are noticed. Other manifestations include lowness of movement, clumsiness, rigidity, leg stiffness, and tremors. While comparing with adult HD, rigidity and seizures are found common, and chorea is reported uncommon in JHD.

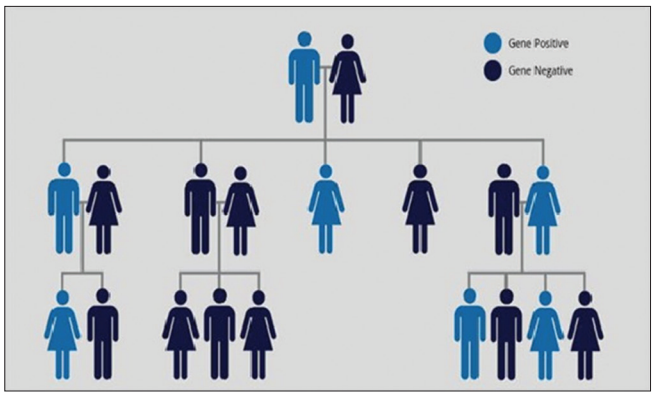

Figure 1: The inheritance of Huntington's disease in a family tree. Source: https://hdsa.org/what-is-hd/history-and-genetics-of-huntingtons-disease/who-is-at-risk/

A few symptoms of JHD

1. Positive paternal family history of HD
2. Changes in oral motor function
3. Behavioral disturbances
4. Decline in cognitive function
5. Stiffness of the legs.

In this article, authors are attempting to adopt bioinformatics tools to diagnose HD precisely using genome browsing tools considering the HTT protein (PDB id: 4FEC) deposited in PDB databank. Further, it is also conceived in this article to explore the small-guided RNA (sgRNA) sequences for HTT gene as a possible guide for genome editing (CRISPR) applications.

Methodology

HD protein and HD gene sequence

HTT gene location using the University of California Santa Cruz (UCSC) Genome Browser

The UCSC Genome Browser is a web-based tool (genome.ucsc.edu) to display a desired portion of a genome followed by a series of aligned annotation "tracks." This UCSC Genome Browser is developed and maintained by the genome bioinformatics group, within the UCSC genomics institute.

The molecular annotations generated by the UCSC genome bioinformatics group display mRNA and expressed sequence tag alignments, gene predictions, simple nucleotide polymorphisms, gene expression, phenotype variation data, and pairwise and multiple nucleotide sequence data. Furthermore, the data pertinent to a region is shown in one

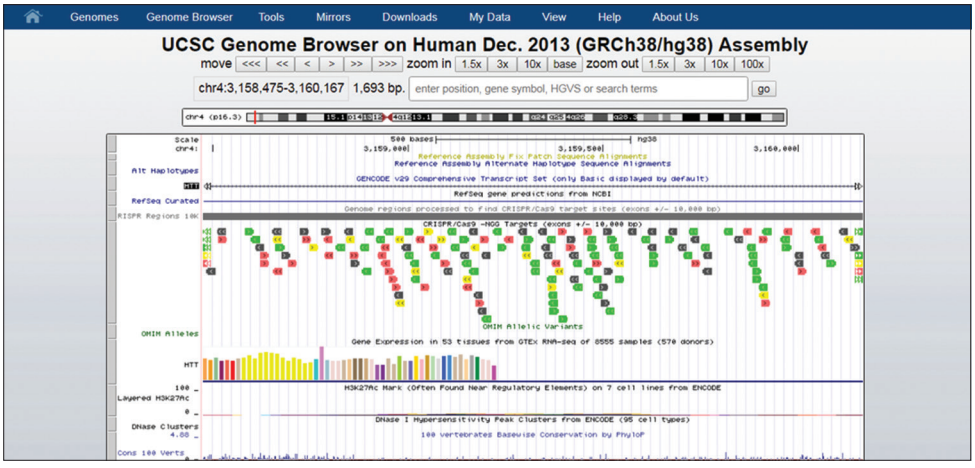

Figure 2: University of California Santa Cruz Genome Browser page displaying zoomed CRISPR targets of HTT gene in green color

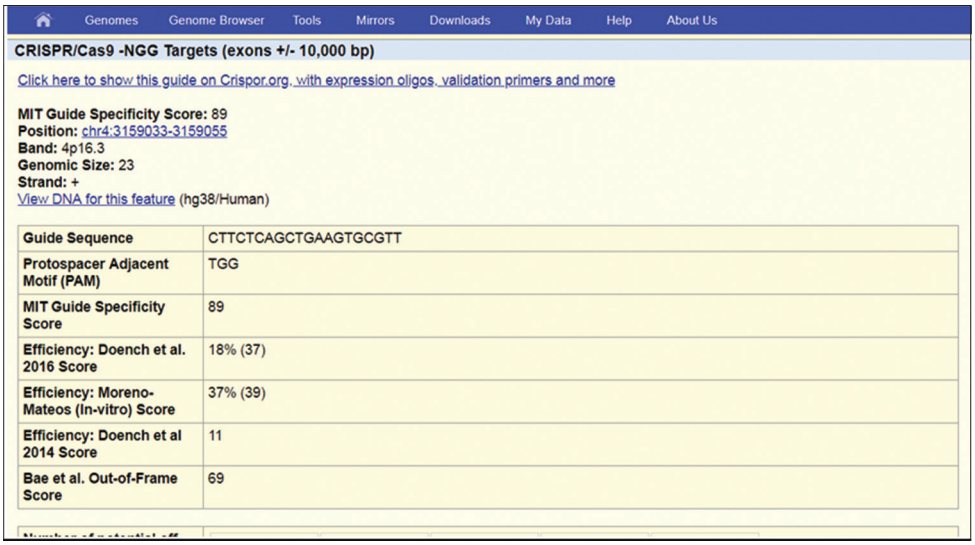

Figure 3: The generated guide sequence (small-guided RNA), PAM, and MIT for CRISPR-Cas9 target (http://genome.ucsc.edu)

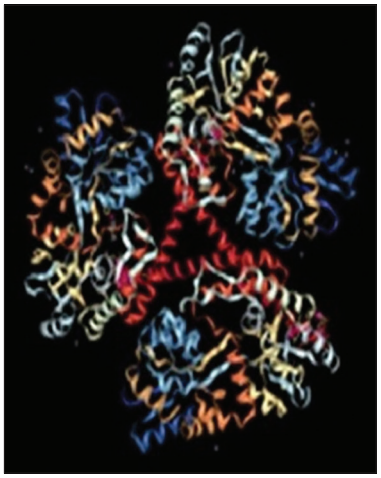

Figure 4: Three-dimensional image of disease-causing protein, HTT structure composed of three domains with alpha-helical coils is predominantly seen. Source: https://swissmodel.expasy.org/

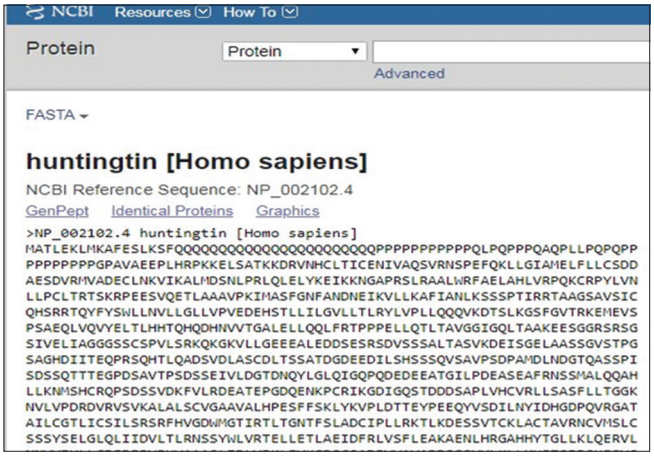

Figure 5: NCBI webpage of HTT protein FASTA format with poly-Q tracking at the start line of the sequence/

window and thus facilitate biological analysis and inference. Hence, HTT gene was retrieved, CRISPR sites were identified [Figure 2], and gRNA sequence was determined [Figure 3].

Guide RNA sequence of HTT using the UCSC Genome Browser

The CRISPR10K of the genes and gene prediction track must be highlighted to "show" (previously it is in hide version). This gives the visibility of CRISPR regions, then zoomed into 10× or 20×, there given many guide sequence in CRISPR10K regions, which on selection gave sgRNA sequence, its matches and mismatches, off-targets, etc., as shown in Figures 2 and 3.

Structural elucidation of HTT protein using Swiss-Model

HTT protein molecular structure elucidation was retrieved through PDB and Swiss Model [Figure 4]. This is a homology-modeling server. This server makes protein modeling accessible to all. FASTA sequences of proteins are accepted by this model for yielding three-dimensional structures of proteins [Figure 5].

Results and Discussion

George Huntington envisaged the autosomal dominant inheritance pattern of HD. However, the modern understanding of the disease remained comparatively dormant and awaited the unfolding of the causative gene, namely, HTT in the year 1983.[3] The location of HTT gene in the UCSC Genome Browser identified its presence at a position 16.3 on chromosome 4 [Figure 6]. The HTT mRNA is browsed through UCSC. The CRISPR sites are viewed [Figure 2] and sgRNA sequences are obtained [Figure 3] through the UCSC Genome Browser. This sgRNA sequence along with Cas9 would be planned for genome editing in future experimental models. It is reported that CRISPR-Cas9-mediated disruption of mutant HTT gene yielded in 50% decrease in neuronal inclusions and appreciably improved lifespan and certain motor deficits.[8] The 3D structure

of HTT protein [Figure 4] retrieved through Swiss-Model has both primary and secondary structures. The Ramachandran plot [Figure 7] for HTT protein derived through online Rampage revealed the recurrent appearance of polyglutamine (Q) [Figure 5] at the Phi (−55–−65) and Psi (120–135) regions.

HD is extensively studied neurodegenerative disorder and the same is available for diagnostics and predictive family genetic testing. There is the possibility of gene-editing therapy in the course of time.[6] Molecular tools and insights given by human genetic research in HD have also reinforced the understanding of yet another neurodegenerative disorders such as Alzheimer disease, with an increased expectation for more ultimate therapeutic approaches.[9] It is known clearly that HD derails a normal social life. Hence, the awareness to understand HD and attention for patients had increased at an alarming rate in the past 20 years.[10] The average duration of sickness of HD is found to be little above 17 years. Hence, one tends to forget the happenings of many years before the onset of HD symptoms.[10] Since the time, the gene was identified in the year 1983, attention was focused on its pathological pathway with the target aim of targeting a therapy.[11-13] Interestingly, the first autosomal dominant disease where pre-manifest diagnosis is made accessible and further, this trinucleotide (CAG) related disease is brought into limelight through DNA sequence analysis.[1,3] This CAG trinucleotide gave us the insight into develop the tools to diagnose through bioinformatic approaches.

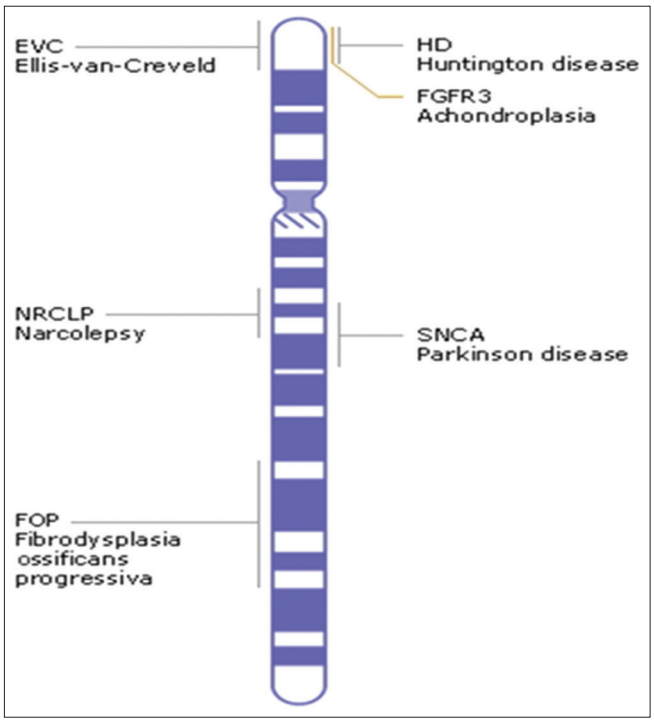

Figure 6: The location of Huntington's disease-causing gene in the euchromatin region of short arm of chromosome 4. Source: https://hdsa/org/what is hd/history-and-genetics-of-huntingtons-disease/history-of-huntinton-disease/

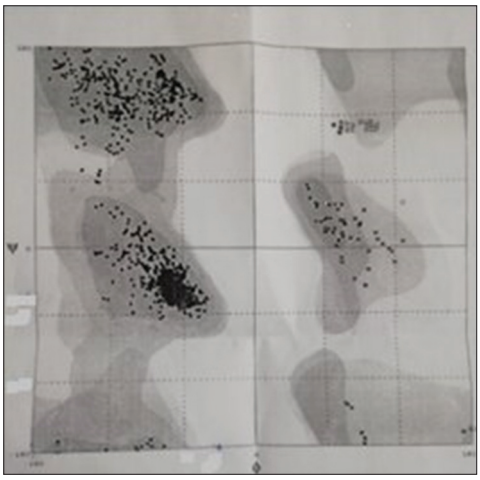

Figure 7: Ramachandran plot displaying the cluster of poly-Q at the Phi (−60–−36) and Psi (−60–−65) regions. The pattern of residues in the plot displays that the HTT protein is alpha-helical predominant/

Online bioinformatic tools such as Swiss-Model, Rampage, and UCSC Genome Browser help in knowing molecular basis of HD and disease-causing protein HTT, and the same invariably assists in creating awareness among health workers.

Conclusion

The present article explored bioinformatic tools to trace the defects in Huntington's gene and design its sgRNA. The location of HTT gene is at a position 16.3 on human chromosome 4. The CRISPR sites of HTT gene were identified and gRNA sequence was determined using the UCSC Genome Browser. This sgRNA sequence along with Cas9 would be planned for genome editing in future experimental models. The Ramachandran plot of HTT protein displayed the cluster of poly-Q at the Phi (−60–−36) and Psi (−60–−65) regions. The pattern of residues in the plot displayed that the HTT protein is alpha-helical predominant. Furthermore, the Ramachandran plot revealed the recurrent appearance of polyglutamine (Q) at the Phi (−55–−65) and Psi (120–135) regions.

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