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

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WEBSITE:  ijhs.org.sa
ISSN:  1658-3639
PUBLISHER:  Qassim University

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.

**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
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. 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. The 3D structure

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**Figure 2:** Identification of CRISPR sites and determination of gRNA sequence using UCSC Genome Browser.

**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/
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. 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. 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. 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. 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. 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. This CAG trinucleotide gave us the insight into develop the tools to diagnose through bioinformatic approaches.

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.

**Acknowledgment**

The authors thank the authorities of Vignan’s Foundation for Science, Technology and Research for supporting students to enroll in research-related projects. The financial support rendered by DST FIST (LS576/2013-C) is greatly acknowledged.

**References**

1. Bruyn GW. Huntington’s chorea: historical, clinical and laboratory synopsis. In: Vinken PJ, Bruyn GW, editors. Handbook of Clinical Neurology. Vol. 6. Elsevier Amsterdam; 1968. p. 298-378.

2. Huntington’s Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable
on Huntington’s disease chromosomes. The Huntington’s disease
 collaborative research group. Cell 1993;72:971-83.
3. Tobin AJ, Bates G, Harper P, Jones L, editors. Huntington’s Disease.
3rd ed. Oxford: Oxford University Press; 2002.
4. Aziz NA, van der Burg JM, Landwehrmeyer GB, Brundin P, Stijnen T,
EHDI Study Group. et al. Weight loss in huntington disease increases
with higher CAG repeat number. Neurology 2008;71:1506-13.
5. Kremer HP, Roos RA, Dingjan GM, Bots GT, Bruyn GW, Hofman MA,
et al. The hypothalamic lateral tuberal nucleus and the characteristics
of neuronal loss in Huntington’s disease. Neurosci Lett 1991;132:101-4.
6. Aziz NA, Swaab DF, Pijl H, Roos RA. Hypothalamic dysfunction
and neuroendocrine and metabolic alterations in Huntington’s
disease: Clinical consequences and therapeutic implications. Rev
Neurosci 2007;18:223-51.
7. Roos RA. Huntington’s disease: A clinical review. Orphanet J Rare Dis
2010;5:40.
8. Ekman FK, Ojala DS, Adil MM, Lopez PA, Schaffer DV, Gaj T,
et al. CRISPR-cas9-mediated genome editing increases lifespan and
improves motor deficits in a Huntington’s disease mouse model. Mol
Ther Nucleic Acids 2019;17:829-39.
9. van Duijn E, Kingma EM, van der Mast RC. Psychopathology in
verified Huntington’s disease gene carriers. J Neuropsychiatry Clin
Neurosci 2007;19:441-8.
10. Wheelock VL, Tempkin T, Marder K, Nance M, Myers RH, Zhao H,
et al. Predictors of nursing home placement in Huntington disease.
Neurology 2003;60:998-1001.
11. Witjes-Ané MN, Vegter-van der Vlis M, van Vugt JP, Lanser JB,
Hermans J, Zwinderman AH, et al. Cognitive and motor functioning
in gene carriers for Huntington’s disease: A baseline study.
J Neuropsychiatry Clin Neurosci 2003;15:7-16.
12. Oster E, Eberly SW, Dorsey ER, Kayson-Rubin E, Oakes D,
Shoulson I, et al. Informativeness of early Huntington disease signs
about gene status. J Huntington’s Dis 2015;4:271-7.
13. Huntington Study Group PHAROS Investigators, Biglan KM,
Shoulson I, Kieburtz K, Oakes D, Kayson E, et al. Clinical-genetic
associations in the prospective Huntington at risk observational
study (PHAROS): Implications for clinical trials. JAMA Neurol
2016;73:102-10.