A Review on CAG Repeat Abnormality Causing Huntington Disease by Genetic Testing

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ABSTRACT

Huntington disease (HD) is known to be as a syndrome which causes nervous degeneration present in the central nervous system. Due to the proliferation of the CAG repeats this disease is occurring which are present in the huntingtin protein. Glutamic acid is coded by CAG repeat. CAG repeats leads to a mutable length of polyglutamine strand which is located at the N-terminus. Inflammation of the neurons and the degeneration of the neurons is a distinctive piece of this progressive disease which forms an assortment of pathological variations within the affected areas present in the brain. The progression of the disease is over the years of about 15-20. The disease is lethal for patient itself and its family. Several symptoms are associated with the disease as depression is aggravating the psychiatric symptom of the disease. Testing relating to this disease is based on having an indication with that to the parent who is already suffering from this disease due the mutable protein.

INTRODUCTION

Huntington’s disease is a disease which is proliferating disease and is a neurodegenerative disease, having a discrete phenotype (Reiner et al., 1997). The disease progresses in the middle age of the diseased patients, but this disease can be initiated at any time of the patient's life. The protein which is mutated named huntingtin in the Huntington’s disease resulting from an extended repeats of CAG length whose location is at the N-terminus. It has been suggested from the evidence that this tail leads to a functional gain which is quite toxic. The mechanisms regarding such disease is not yet clear, information has been provided by transgenic animals about HD factors which cause the disease (Sathasivam et al., 1999). Huntington’s disease has been called a degenerative disease of the neurons.

In the Western world occurrence of the Huntington disease is 4-10 per 100,000 in which many people are at the threat of the disease (Bonelli & Hofmann, 1990). Years before a motor diagnosis is made, the symptoms of the HD emerge from the biological changes of the neurons, due to which the emergence of the huntington disease has been occurred. Chorea is the symptom noted earlier in this case of HD but more kerbing is the motor injury and in coordination (Bates & Hockly, 2003). The damage of the neurons is most significant in Huntington disease, but damage of the cells of the neurons in certain brain's part is the most significant due to which debility in the functions of the motor neurons are more likely to be occurred (Arnulf et al., 2008). The problem occurs due to malfunctioning of a single gene. CAG expansion causes the disease on chromosome 4 (Aziz et al., 2007). For the development of the neurons huntingtin is very important. The protein is mutated in Huntington’s disease which is causing many problems regarding this disease because proper functioning is not done. Through many pathogenic mechanisms, the disrupted protein huntingtin leads to the disease. There is 50 % ratio that this disease could be transmitted in the next generation. The generations are affected in this disease (Cattaneo et al., 2001).

CAG REPEAT

A little proliferation of the repeats does not cause the disease. Irregulation or expansion occurs, if the repeats are more than 36, there will always be Huntington disease if CAG repeats exceeds more than 40 (Bateset al., 2002). Between the 36 and 39 CAG repeat lengths abridge penetrance is shown, which shows that some people with these lengths will show the progression of the disease and some will not; those in which the progression of the ailment is not shown, develops the later inception of the
disease (Fig. 1). This disease does not occur in between little proliferation of repeats but may develop in future generations into the pathogenic range (Bruyn 1968). This causative agents and the prompting factors are not identified, with no superficial history of family in which the disease progresses. In 6-8% of the disease apparent sporadic Huntington’s disease arises, or due to sudden or unknown paternity and also by parent dying earlier, before the outcomes of the disease are shown (Bradford et al., 2010). The length of the proliferated repeats depends on the age factor of the diseased person so it can be said that if the more proliferation or the mutated protein is then there will be the more chances that earlier will be the outcomes shown (Bennett et al., 2007). About fifty to seventy %of the chances of the mutated protein in HD are shown and about that outcome it should not be foretold by experimentation before definite approval is given (Walker 2007).

**Fig1. The proliferation of the HD is 4-10/100 000 in the western world. It arises at 40 years of age and within 15-20 years of the progression of the disease, death may occur.**

**CLINICAL GENETICS**

It has been said that the position of the mutated gene is on the Chromosome 4 and has been analysed through various testing and expanded trinucleotide repeats are linked with it (Cattaneo et al., 2005). CAG repeats are present on normal alleles, but the disease is wholly penetrant when the repeats reach to a length of 41 or more than this. Repeats of 37 or less than this are not connected with the disease, from 36-40 repeats happening of incomplete penetrance occurs (Caviston & Holzbar, 2009). CAG repeats number has accounted to about 60% of the deviation in onset of the aging; the remainder is represented by the modification of the genes and environment. Insecurity on the replication occurs when the trinucleotide CAG repeats exceeds 28, because of the proliferation of the repeats, most unpredictability leads to extension (73%), it can be more shown or proliferation of it is more seen in case of males as compared to the females on replication large expansions of CAG repeats specially in males (Chattapadhayay et al., 2005). In consecutive generations the symptom of the disease can be shown at the early stage of the life of the HD suspected person and with juvenile onset signs the inheritance from parents in children. Due to the proliferation of the repeat length from twenty-eight to thirty-five, a different outcome of the disease are shown, usually on the paternal side (Rubinsztein 2002). It is not only limited to the germ line mutation but is also shown in the somatic cells in HD. This is not matter of complication in most cases because it is not inherited can remain in one individual because through various experimented analysis it has been shown and this finding can add to selective vulnerability.

If the two patients are identical twins and share the same genetic makeup then outcomes of the disease in one patient will also show the outcomes in the other patient as well but clinical different
phenotypes have been shown in certain cases (Djousse et al., 2004). A homozygous case of the sickness has displayed no significant alterations of the expression of the disease (Bonifati & Kishore, 2007).

**PRINCIPLES OF PATHOGENESIS**

The mutated protein is much proliferated and is having the length of the mutated one containing the 50 amino acid which are called as Heat. Composition of these repeats is 2 helices which is giving the shape just like a pin of the hair whose core is tangled in further again more loop like structure (Difiglia et al., 2007). The mutated protein is bounded with other protein also from the N-region, it is showing the function of the protein to show its interaction with the neighbouring proteins as well. HTT also does widespread post-translational modification. The other functions performed by the mutated protein in HD are not yet understood (Sapp et al., 2001). HTT is having a role in vesicle transport which shuttles into the nucleus, and can control the gene transcription and RNA transferring. It has been analysed that there will be the more progression of the disease, if the toxic components are produced in HD, because this will interfere with the already proliferated disease and is shown by experimentation performed in animals by taking them as a model for the research so that various outcomes could be analysed because this all has been done due to the mutated protein (Henley et al., 2009). The RNA in this case is posing the harmful effects or the toxicity due these alternations the functional ability of the protein is not done so there will be the need of the RNA with opposite strategy. Through the genetic engineering the effect of the defected protein is controlled and is replaced with the corrected one. For example by the addition or deletion of the genes it can be done (Wild & Tabrizi, 2007).

Heat repeats are the composition of Human HTT. At the N-region there is present segment of glutamine. Proteolysis through the caspases including the caspase six as well as the enzymes which are undergoing lysis produces toxic components (Fig. 2). It is not yet identified that what can be the size as well as about the degrading enzymes and toxicity of HTT’s. Amino breaking sequences are the IVLD and NLPR. NES is the nuclear exporting signal (Difiglia et al., 1997). With the progression of the Huntington disease inflammatory proteins which complement the clusterin and complement proteins is regulated in the diseased patients. The evidence from the biochemical, post-mortem tissue and animal models showed that proteolysis of HTT is a key to highlight the toxic effect and other harmful effects of the HD. Labelling of the inclusions with the antibodies to epitopes which would be near the N-terminus not near the C-terminus, have shown that that the inclusions contains HTT species which are truncated in which the protein’s N-terminus is included (Shin et al., 2005).

![Fig2](image-url) **Fig2.** HTT of the human is composed of the repeats called HEAT. On N-terminus, poly Q stretch is located. Caspase 6 carries out the proteolytic cleavage. NES is the nuclear export signal. Amino acid cleavage sequences are IVLD &NLPR.
NEUROINFLAMMATION AND NEURODEGENERATION IN HD

Neuroinflammation is performing the two functions because besides giving the benefit it is also posing the harmful effects as well. The acute form of it is very beneficial in the removal of the damaged cells which could pose the harmful effect (Jones 2002). This is the most helpful in removing the toxicity in the central nervous system. The chronic form of it is associated in posing the harmful effects to the neurons. There is not only extended proliferation of the repeats but there will be the more cytokines to be released in it and also there can be the increased superoxide release as well as the production of nitric oxide. This effect extends the BBB which is resulting in the improper filtration of the macrophages in the specific areas of the brain which is then resulting in the produced toxicity inside the brain which is leading towards the nervous breakdown and its damage in HD patients (Kremer et al., 1991). Neuronal death which is caused by toxic mutant Htt leads to the inflammation present at the N-terminus, In the cortex of the brain, the Htt is found which is mutated and due to different chain length in it (Langbehn et al., 2004). Inflammation or toxicity is occurring due to the presence of the Htt gene, if the inflammation has been brought about by mutant Htt which is expressed in the milieu or by inflammation due to the activated microglia (Fig. 3). For an immune-privileged brain, there can be the overexpression of the molecules which is abnormally secreting the cytokines, the more toxicity and inflammation in HD is known to be occurring due to the secretion of cytokines in the central nervous system as well as in the peripheral nervous system (Shin et al., 2005).

The cells of the microglia are in the state of rest and are equally effecting its environment. The brain is protected by the BBB by means of the surrounding environment of the cell, some cells of the immune system, including the pathogens as well as the other material (Schilling et al., 2007). In Huntington disease, the proliferation of the Htt is occurring due to the degeneration of the neurons and in result
the resting state of the cells is over and they becomes active in order to response to that stimulus and cell’s mechanism to response to the stress is active an cells starts the phenomenon of the phagocytosis as well as the secretion of the hormone called cytokines is occurring (Fig. 4). Neuroinflammation is occurring due to this hormone (MacDonald et al., 1999). This is performing the important function of degrading the BBB and also immune system activation and its response to the external stimuli is occurring. The brain cells are undergoing their degeneration due to the cells called microglia which causes the expression of the Htt (Wild & Tabrizi, 2007).

**AETIOLOGY**

Due to proliferation of the CAG repeats HD is occurring and this sequence is known to pass from one generation to another because the gene can be inherited. Htt protein is coded, and is coded no exon 1, this is showing the pathway of CAG (Paulsen et al., 2006). This type of it comprises a CAG recurrence, which codes for a section of polyglutamine which is present in the protein in the range of six to twenty-six. The repeats linkages are occurring after every thirty-six repeats (Walker 2007). Appearance of the disease or the mutated protein, if the proliferation is occurring or it prolongs to the repeats of about 40. If the length of the repeats are from thirty-six to thirty nine then there will be the little symptoms of the disease to be shown in this case. The repeat length from twenty-nine to thirty-five is unbalanced, changing in reproduction which shows that these alleles are prone. It is transmitted or it can occur mostly in the males (Purdon et al., 1994). As the length of the repeats is proliferated the more will be the chances of the occurrence of the disease. The disease will show its symptom at the early stage of the life if there will be the more proliferation of the repeats. When the diseased person will reach at the age of twenty then under such symptoms showing disease, the disease is known to be as juvenile Huntington’s disease (JHD), the repeat often surpasses. No indication about the preliminary symptom, course or the illness period is given as the repetition of the repeats is controlling the seventy % of the alternation at every step of the age, during the prevalence of the HD (Azizet et al., 2007). Due to the mutation or abnormality in the repeat length, the dramatic loss of weight in the patient of HD is shown. Synaptic Function has been shown by the normal wild-type Huntington protein, and is essential after the embryonic stage has been developed and is also providing the protection against the apoptosis because it has been involved in the self-defence mechanism in response to that mutation in HD (Cattaneo et al., 2001).

It has been proved from the experimental trials that several changes are induced in the body of the patient in HD as well as several alternations also results in the malfunctioning of the various mechanism occurring in the patient means debility to various functions occurs (FrankCannon et al., 2009). This alternation or loss of various functions has been studied on the basis of experimentation including mice models which are almost about ten on which experimentation has been done. As it is clearly observed by studying at the level of the nucleus as well as the cytoplasm, but their work is not yet identified by studying (Djousse et al., 2004). The inclusions can be in the CNS divided into many parts. The complete toxicity which it poses, which effects, the functioning of the brain, which has the specificity with the most damaging in the neurons, it is best, understood regarding such perspectives (Roos 2010).

**GENETIC TESTING**

Confidentiality and Consent

Before and after predictive testing, strict confidentiality has been observed according to internationally agreed guidelines (Trottier et al., 1994). Before the testing is done so there is the need to take the confidentiality from the person which is going to be examined. There should be the inclusion of the siblings in it. But if after testing the symptoms of the HD are shown, the patient should not be clearly informed about that except its family members because it might create complications (Cattaneo et al., 2001). If the patient insists on then signed consent should be taken from the patient himself because it is obligatory, before the diagnosis of the disease is preceded. If the patient has an undecided behaviour then in this case the testing consent must be taken from the person and is approved by the patient which can be its representative (Vonsattel 2008).

**Having Children**

It is very important, that at what stage they should be having a child or not which is always a danger of the progression of the disease or the proliferation of the HD. So in this case of the risk different
testing are performed, or is chosen by the minority, ensuring that having the expanded HTT gene chance will be about < 1% (Wexler &Venezuelan, 2004).

**Prenatal Testing**

By means of sampling of chorionic villus done between the 11-13 weeks of pregnancy, prenatal testing is carried out. It is important to have pre-test counselling in potential parents so that they could terminate the pregnancy if he developing foetus is having expanded Htt gene, otherwise predictive testing will be done (Fig. 5). Linkage analysis is done for prenatal testing (Langbehn et al., 2004).

**Preimplantation Genetic Diagnosis**

For the test of the mutant HTT gene, there is the need to create the embryo in which normal procedures of in vitro fertilization has been use (FrankCannon et al., 2009). There is the need to implant the embryos which are Unaffected. There will always be the variation in the success rate; live birth rate will be 1 in 5 cycles (Schilling et al., 2007).

**Segregation Prenatal Trying and Preimplantation Inherited Analysis**

Connection practices have been used, CAG expansion analysis is not needed, gene status of the potential parents is not found. Foetus will be having the 50% chances of developing the disease as the parents were having. So, there is the need for the termination of the pregnancy at the risk of the 50% chance (Trottier et al., 1994).

![Fig5. Linkage analysis usage for prenatal testing. Linkage markers are present on chromosome 4.](image)

The toxic effects and the direction of the HD are not understood. The crucial steps are difficult to be finding out in this case. The screening approaches should be considered regarding the HD (Bonelli & Hofmann, 1990).

**Clearance of Mutant HTT, Transcription, and Translation**

In neurodegenerative disease, the therapeutic strategy is to reduce amount of pathogenic protein, Huntingtin. It is an inducible transgenic system in order to stop the production of mutable protein (Caviston & Holzbar, 2009). Housekeeping promoter is present in the HTT gene, so that its regulation for the production of mutable protein could be effected. Small interfering RNA (siRNA) or antisense oligonucleotides are targeted or inserted into the brain in order to stop production of mutable protein (Fig. 6). The experiment to shut off the mutable gene is being shown by performing experimentation on mouse (Wild & Tabrizi, 2007). Compensatory mechanisms are present inside the cell, like if any
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foreign that gains entry inside the cell it is removed or antibodies are produced in order to remove it, so that unfolded and abnormal proteins could have been removed, there can be many methods for its removal as one is the ubiquitin-proteasome scheme (Rubinsztein 2002).

![Diagram](https://via.placeholder.com/150)

**Fig6. HD using compensatory mechanisms for cellular pathways.**

The mutated gene undergoes the complication in credit of target as well as the degradation of the cell parts. Another method which is applied for the degradation of the mutated protein is by increasing the action of the chaperons, which is performing the function to identify the mutated protein which is not properly folded and then again folds the protein and gives it a proper shape (Kremer et al., 1991).

**CONCLUSION**

CAG repeats abnormality is causing HD, it’s predicted that if the length of the repeats is proliferated then there will be the more chances of the outcomes of the HD. HD is a lifetime syndrome for the individual as well as its family means it is transmitted to the next generation. The mutant protein called as Htt wholes is causing the degeneration of the neurons as well as it is also producing the toxicity leading to inflammation. Genetic testing has given the awareness regarding the disease, that how the disease is proliferated and how it can be prevented.

**REFERENCES**


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