ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 7, pp.5175-5179, July, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

CAG EXPANSION LENGTH CORRELATION WITH THE RATE OF CLINICAL PROGRESSION IN HUNTINGTON'S DISEASE

[1*]Al-Kamali, F., [1]Osman, F and [2]Gomaa, R

[1,2]Department of Biotechnology, University of Modern Sciences, United Arab Emirates

ARTICLE INFO

Article History:

Received 14th, June, 2015
Received in revised form 23th, June, 2015
Accepted 13th, July, 2015
Published online 28th, July, 2015

Key words:

Huntington's disease, CAG repeat, length polymorphism, genetic diagnosis, neurodegeneration.

ABSTRACT

Huntington disease (HD) is an autosomal dominant, monogenic neurodegenerative disorder.Huntington's disease is caused by a trinucleotide repeat expansion of CAG in IT15 gene at locus 4p16.3.The purpose of this study was to identify the relationship between CAG repeat length and the progression of Huntington's disease. It is commonly reported that CAG repeat length is related to the age of onset of the disease. MRI measurements of early onset patients revealed the most rapid rates of atrophy and cognitive decline compared with those who developed symptoms during middle age or more advanced age. Further analysis suggested that patients with long repeat lengths (≥47) had an earlier age of onset and that the younger group of patients displayed significantly increased decline in both cognitive and neurologic functioning over the 2-year interval period of follow up than those with shorter repeats. These findings suggest that the CAG repeat length may influence or trigger the onset of HD but other factors might contribute to the progression of illness and the pace of neuronal degeneration.

INTRODUCTION

Huntington's disease is caused by a trinucleotide repeat expansion of CAG in IT15 gene found in the short (p) arm of chromosome 4 at position 16.3 (4p16.3). (Morrison, 2013) This particular expansion of CAG length results in a defective protein called Huntington protein, for which the exact role is unclear, but it functions in affecting the nerve cells. (Cattaneo *et al*., 2005) Neurodegeneration of the brain gets gradually progressive over time and can affect movement, behavior and cognition of the individual. (Bates, 2005)The main hypothesis is to correlate the length of CAG number of repeats to the progression of the disease. This CAG expansion is normally repeated 26 times or less in healthy individuals. In case of diseased individuals, the number of CAG expansion is more than 40. The individuals with intermediate length of CAG, between 27 and 35, might develop clinical manifestation of the disease and Ha *et al*. (2012) study was aimed to identify the main clinical characteristics associated with this CAG number of repeat range. The length of CAG repeat therefore substantiated to be a novel biological marker for the diagnosis of Huntington's disease. However, according to Xuereb *et al*. (1996), genetic analysis is not enough when it comes to rare cases of Huntington's disease patients with normal CAG repeat expansion and further neurophysiological analysis has to be taken in consideration in order to detect the disease. One of the latest studies qualified neuroimaging as a tool due to its' exceptional ability to identify the progression of the disease. Furthermore, it can reliably distinguish clinical heterogeneity and subtypes that have the potential to react to treatments differently. According to data collected from all studies included in this review, CAG expansion repeat length strongly correlates inversely with the age of onset of HD. Rosas *et al*. (2011) described that the length of CAG repeat was longer with more rapid atrophy rate and cognitive decline of the brain have been observed in young adults age of onset. Middle-age onset and older patients have shorter CAG number repeat with slower progression of the disease.

METHODOLOGY

***Subjects*:** Fifty blood samples were obtained from HD patients in Kieburtz *et al*. (1994) study. The study did not specify age of the participants. On the other hand, 46 patients enrolled in Brandt *et al*. (1996)study. The chosen subjects were mildly to moderately affected and the study was over three occasions with a 2 year-interval. Patients were divided into two sections. Twenty one patients with 47 or more repeats were categorized as long mutations and the rest twenty five patients with short mutations ranged from 37 to 46 repeats. Violation of the protocol of this study occurred because six patients were identified to have 36 or less of CAG repeat length. A larger number of patients, however, joined in Xuereb *et al*. (1996) research reaching 268 patients but this study mainly focused on

Corresponding author:* **Al-Kamali, F
Department of Biotechnology, University of Modern Sciences, United Arab Emirates

the neuropathlogical comparison and genetic testing as diagnostic tools of HD. Moving on, participants in Ha *et al.* (2012)were categorized in accordance of the number of CAG repeats into normal with CAG_n of 26, intermediate with CAG_n between 27 and 35, and HD with 36 and more of CAG number of repeats. Comparisons between intermediate and normal group was the main focus in this study since the principal objective was to evaluate the clinical characteristics in people with intermediate number of CAG repeat. Meanwhile, about 1985 patients were examined in the Ha *et al.* (2012) study achieving the highest number of subjects comparing to other studies mentioned in this review. Although the number of patients was large, 50 of them had intermediate CAG repeat included in the study. That is, 2.5% of total three categories in particular.

Twenty-two patients with symptomatic HD [stage I and II, as defined by the United Huntington's Disease Rating Scale (UHDRS) and Total Functional Capacity (TFC) scale] enrolled in Rosas *et al.* (2011) study and were sorted into three sections based on the age of onset. "Young" who developed motor symptoms at the age less than 40 years old. "Mid" who developed motor symptoms between 40 and 55 years old and "Old" with motor symptom development with age more than 55 years old. Enrollments of subjects in the COHORT study for those who were not genetically tested but have first-degree relatives are included. Along with people who had their grandparents diagnosed with HD. Moreover, even individuals who did not have genetic mutation but have positive family history were included in Rosas *et al.* (2011) study. CAG number of repeats was between 40-55 in this study.

Samples: The majority of samples used in most studies were taken as blood in order to perform PCR to detect the CAG expansion. In Ha *et al.* (2012) study, DNA was extracted from two types of samples, whole blood and lymphocytes. If unsuccessful match of the results was observed, both tests were repeated until the result values were matching. Pinpointing how cautious this study was in association to the quality of the DNA. In contrast, the sample in Xuereb *et al.* (1996) research used was frozen half brain tissues from dead patients putting the results concluded in doubt since there was a difficulty in extracting the DNA from such samples. Samples preserved and used in this study were not checked for quality and the duration of the disease was not noted and tested. Moreover, small amount of samples were successfully amplified. Therefore, DNA extraction method was not completely successful raising the potential of bias results. Early studies used PCR reaction as their main method for identifying the number of repeats.

Diagnostics Instruments and Assessments: All studies involved clinical questionnaires for the patients. Each subject underwent formal motor examination in all studies. Subjects were scored according to the motor component of the United Huntington's Disease Rating Scale (UHDRS) in Ha *et al.* (2012)study. Furthermore, Kieburtz *et al.* (1994.), Rosas *et al.* (2011), and Brandt *et al.* (1996) included the use of the Total Functional Capacity (TFC) scale in relation to age and number of CAG repeat. Additional examination tests including Quantified Neurological Examination (QNE), Mini-Mental State Examination (MMSE), Vonsattel grading system, HD activities of daily living (HD-ADL) and neurological battery were all used in Brandt *et al.* (1996) research. Consequently, offering a decent amount of evaluation data collected that will markedly help with the results analysis. Even if the results were found eventually variable, principal component analysis procedure was used to reduce variable results.

The latest research, Ha *et al.* (2012), methodology, included MRI analysis to test the HD progression of patients with the same stage of the disease but in different age onset. Body Mass Index (BMI) was also included to measure the weight of the patients. Measurement control was specified for the assurance for more accurate registration method for rate determination. Baseline visits involved standard neurological and physical examinations that were categorized in four domains including motor function that consisted of 31 items, in addition to cognition, behavior and functional capacity. Follow-up visit comprised general MMSE to BMI examination. Genetic diagnostic methods included electrophoretogram and regular PCR amplification of the IT15 region contains CAG repeat expansion. The amplified DNA fragments were separated on polyacrylamide gels by gel electrophoresis technique and the results were obtained of the CAG repeat number. Family pedigree was also considered in some studies.

RESULTS

Among the earliest studies, there was little evidence that supports the potential for the relation of CAG repeat length with the illness progression. Although the length of CAG repeats revealed a strong inverse correlation with the age at onset of Huntington's disease, there was no such relationship between the number of CAG repeats and the rate of clinical decline as mentioned in Kieburtz *et al.* (1994.) These findings suggest that the CAG repeat length may influence or trigger the onset of HD, but other environmental, neurobiological, or genetic factors contribute to the progression of illness and the fundamental pace of neuronal degeneration. The TFC initial evaluation was 9.2, and the TFC at final evaluation was 6.8 with a decline of 0.5 TFC units per year and only four patients showed improvement after treatment of the disease giving negative TFC units. There was no relation between number of CAG repeats and TFC units aka, conversely, a strong relationship between the age of onset and CAG repeat length was detected.

Brandt *et al.* (1996) results verified that individuals with longer CAG_n developed illness progression twelve years earlier than those with shorter CAG_n. Since this study divided patients in two categories based 'Short' and 'Long' repeat group. Patients with longer CAG expansions were younger than those with short repeats but did not differ in the severity of neurologic or cognitive impairment. Over time, both groups showed increasing in neurologic, functional, and general cognitive impairment. Nevertheless, the long-repeat group displayed significantly greater decline in both neurologic and cognitive functioning over the 2-year follow-up period. Total score of the Quantified Neurological Examination (QNE) ($F[2,861 = 8.14$, $p < 0.001$), cognitive factor 1($F[2,861= 3.24$, $p = 0.044$) and Motor Impairment Scale ($F[2,86]= 4.35$,$p = 0.016$.) The substantial connection on the total QNE and the Motor

Impairment Scale indicate that patients with longer CAG repeats had a decline in both neurologic and cognitive function over the 2-year interval than did patients with shorter repeat length. (**Figure 1.**)

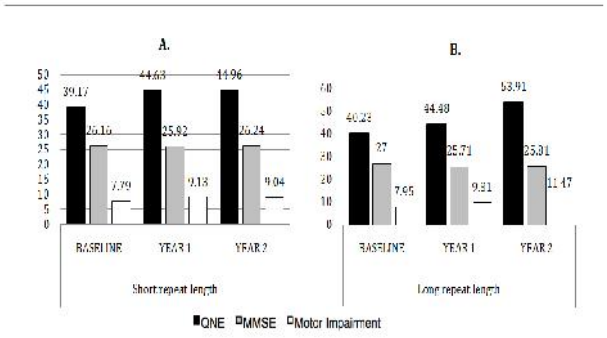
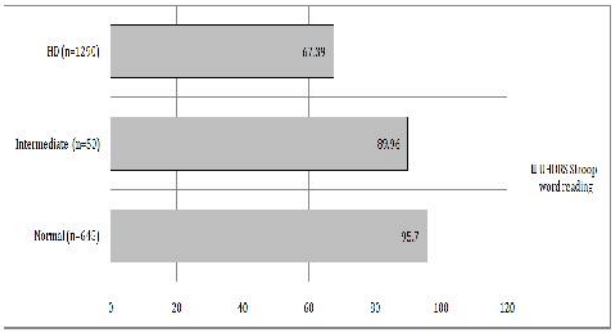


Figure 1. (**A.**) Patients who were categorized as short mutations (37 to 46 repeats) showed less deterioration in Quantified Neurological Examination QNE, The Mini Mental State Examination (MMSE) test, and Motor Impairment test than (**B.**) patients with longer mutation (≥47).

As mentioned earlier, the use of frozen half brain as a sample in Xuereb *et al.* (1996) study established only 63% success of the DNA extraction. That is a 169 out of 268 patients. Abnormal repeat expansion was detected in 99% of patients. One exceptional result of a patient with 22 repeats showed typical clinical illness and positive family history. That patient with normal number of CAG repeat scored grade 1 in consideration of Vonsattel grading system. Meanwhile, the other 112 patients who graded between 2-4 did not show any significant difference in regard to the number of repeats. Thus, there was no correlation between the severity of the disease and number of CAG repeats. In contrast, three patients with CAG expansion and positive family history had no changes whatsoever regarding morphological changes. Nevertheless, six patients with no observable expansion of CAG and negative family history were diagnosed with Huntington's disease as reported by the neurophysiological analysis. Different scores were obtained regarding the United Huntington's Disease Rating Scale (UHDRS) scale in normal and intermediate groups at baseline in the exclusive study of Ha *et al.* (2012) Intermediate group reported one suicide attempt. All rates of United Huntington's Disease Rating Scale (UHDRS) scale suggested disease progression in intermediate group. 333 of patients were excluded because CAG repeat length was not determined.



**n indicates number of patients.*

Figure 2. Unified Huntington's Disease Rating Scale (UHDRS) Stroop word reading test demonstrates the rapid reduction rate of the test among different groups as the length of the repeat increases. Normal (26 repeats), intermediate (27–35 repeats) and HD (>36 repeats.)

Educational wise, no observable numerical difference between HD and intermediate group. Marriage alongside with employment was significantly higher in intermediate group compared to HD group who had much lower outcome. In case of Body Mass Index (BMI), no recognizable variance between normal and intermediate groups was detected. HD group, however, showed a low BMI stating that weight loss symptom relates to the length of CAG repeat. Additionally, Unified Huntington's Disease Rating Scale (UHDRS), Word Reading (WR) along with Maximal Dystonia (MD) and other related sections scored much differently in intermediate group in comparison to control (normal) group.

The preferment of 'Intermediate' category in this study is between 27 and 35 number of CAG repeat. Even if studies consider the disease will develop symptoms at 36 and above CAG repeat range, this article verifies that some patients with intermediate CAG repeat (27 to 35 repeat length) reveal symptoms of the disease. This raises the potential to consider the increase size of allele length in an intermediate individual, even if a subtle abnormality observed, might cause or intend to cause progression of the severity of the disease. Although this was a longitudinal study, results of baseline were given without any follow-up data observed. (**Figure 2**)

In Rosas *et al.* (2011), all patients were at the same stage of disease. After a year, younger patients suffered from more severe progression of disease. Significant differences were observed in the cortex, white matter and subcortical structures. Young people who developed symptoms had more rapid rate of brain shrinkage than older patients. Motor symptoms of people younger than 40 years old were associated with more rapid atrophy related to older patients. 55 years old patients had a slow rate of progression of the disease. Moreover, by using MRI, the rate of clinical progression of younger patients advanced the rate of older age onset patients. CAG number of repeat did not influence much with the rate of atrophy. Most variable results were obtained from cortical atrophy. Parietal cortex, sensorimotor and posterior areas showed noticeable topological changes. In contrast, no changes were observed in the basal ganglia area. CAG repeat length and age played an important role in the changes in some cortical regions apart from other regions that were not significantly affected.

Moreover, CAG repeat length did not influence the rate of progression of the disease as much as the age did. Other unknown factors can manipulate the progression of the Huntington's disease and follow-up hypothesis must take into consideration the unknown factors that might influence the progression of the disease. Rapid thinning in the cerebral cortex regions of young group was more evident than any other group.

Table 1 Young group indicated to have the longest CAG_n repeat length and the most rapid thinning rate in cerebral cortex area.

	Young group (n=6)	**Mid-aged group (n=9)**	**Old hd (n=8)**
Age	33.1	49.3	62.2
CAG repeat	52.2	43.8	42.1
Cerebral cortex thinning rate	3.6%/ year	1.55% per year	1.77%/ per year

It has been observed that 3.6% of whole brain was reduced in one year interval in young group. Mid-aged group had less progression of the disease reaching 1.55% per year of whole brain thinning while Old age group showed a rate of reduction of 1.77% of reduction. (see **Table 1.**)

In case of gray and white matter loss, young aged group scored higher rate of reduction comparing to the mid and old. Hence, the rate of progression of the disease and cortical thinning were rapid in young group. In term of surface-based maps of sensorimotor and middle frontal on the right hemisphere regions, Young group rate of loss was 8% and 5% respectively every year. Mid-aged group scored less figures approximated with 3% and 5%. The rate of thinning of old-aged group, however, was much less than both groups reaching 3% only. Furthermore, Corpus Callosum rate of thinning in young group was generally faster than mid and old patients within all regions.

No substantial change in rate of basal ganglia atrophy suggested in all groups regarding subcortical structure. On the other hand, floor effect was suggested when the rate of caudate atrophy was much slower in young group compared to the other two groups. Though, baseline volumes in young group was 40% smaller than mid (25%) and old (20%). On average, for those areas that were correlated, each unit increase in $CAG_n$was associated with a 0.3% faster rate of thinning. Although, the length of CAG number of repeats did not relate to the rate of volume loss regarding subcortical structure caudate, putamen and thalamus. There was a relationship between cross-sectional caudate volumes at baseline and CAG_n. 11% of cortical regions correlated with the length of CAG number repeats showing progressive thinning suggesting that cortical thinning is not strongly dependent on the CAG_n.

DISCUSSION

This review paper correlated the age of onset, and rate of progression of Huntington's disease with the CAG_n repeats in IT15 gene on chromosome 4. In the past, it has been thought that CAG repeat length strongly impacts on age of onset but not with the progression of the illness according to Kieburtz *et al*. (1994). However, this research was conducted long time ago. Moreover, the results provided were not coherent enough to support the justification in addition to the weak amount of information proposed. Recently, results of several studies indicated the possibility of CAG repeat length to interact with the severity of the disease. Furthermore, severe function impairment of patients with long repeats (≥47) over the 2-year interval compared to those with shorter CAG repeat was reported. Hence, the length of duration of the disease appears to be more progressive in patients with longer CAG expansion. (Foroud *et al*., 1999) Although that was completely dependent on Motor Impairment Scale as an indicator and few patients were enrolled, putting Brandt *et al*. (1996) study's assessment methods and results obtained in limitation, other new studies confirmed the hypothesis to be factual. Although the Total Functional Capacity (TFC) scale is considered as a principal measure for research development regarding Huntington's disease, Downing *et al* (2013) found that World Health Organization Disability Assessment Schedule (WHODAS) 2.0 scale to be more underlying considering statistical measures. It is a measure used daily to extent health conditions of patients with neurophysiological disorders.

The results of Ha *et al*. (2012) study obtained to verify whether individuals with intermediate CAG expansion length was one of the crucial hypothesis in order to validate if the CAG expansion does certainly affect the progression of illness. The number of intermediate group was negligible in comparison to the normal group and this limited participation of intermediate group was a drawback because it permits consequential conclusion of the results. Errors in results might accompany when a substantial amount of data was observed. Additionally, despite that United Huntington's Disease Rating Scale (UHDRS) is a well-known assessment in clinical trails, it is not sensitive enough for early stage patients. However, abnormalities were only addressed in the intermediate group. Thus, inaccuracy is less likely to occur. Although this was a longitudinal study, results of baseline were given without any follow-up data observed. Subtle evolution of the disease suggested on intermediate group, which gives the possibility of CAG length relation with disease development. This raises the potential to consider the increase size of allele length in an intermediate individual, even if a subtle abnormality observed, might cause or intend to cause progression of the severity of the disease.

In case of neurodegeneration assessment of the disease using MRI as a lead tool for observation, there was no relationship between CAG_n and the rate of volume loss in any subcortical structure, but Rosas *et al*. (2011) did find a relationship between the cross-sectional volume at the baseline scan and CAG_n. On average, for those areas that were correlated, each unit increase in CAG_n was associated with a 0.3% faster rate of thinning. 11% of cortical regions correlated with the length of CAG number repeats showing progressive thinning. Advocating that cortical thinning was not strongly dependent on the CAG_n. However, all patients were on the same stage of disease when conducted in baseline. After a year, younger patients with longer CAG expansion suffered with more severe progression of disease. Rates of regional brain atrophy were influenced by the age of onset of HD symptoms and are only partially explained by the CAG repeat length. These findings suggest that other genetic, epigenetic and environmental factors play important roles in neurodegeneration in HD. Other unknown factors can manipulate the progression of the Huntington's disease and follow-up hypothesis must take into consideration regarding the unknown factors that might influence the progression of the disease. Regarding the methodology of detection of the disease, genetic analysis is not enough when it comes to rare cases of Huntington's disease patients with normal CAG repeat expansion and further neurophysiological analysis has to be taken in consideration in order to detect the disease. The reason behind that is, in Xuereb *et al*. (1996) study, the sensitivity and specificity of neuropathological examination of Huntington's disease were confirmed. Attaining 100% specificity when using this method and never compelling possibility of false-positive results of diagnosis. The genetic diagnosis is not accurate, perhaps, when rare occurrence of mutations taken into account. Hence, one of the unknown factors that are involved in the follow-up

hypothesis will be the several subtypes of mutation associated with HD.

Critical point regarding Xuereb *et al*. (1996) study, samples preserved and used were not checked for quality and the duration of the disease was not noted and tested. The sample used in this study was frozen half brains from dead patients putting the results concluded in doubt since there is a difficulty in extracting the DNA from such samples. Moreover, small amount of samples were successfully amplified. Therefore, DNA extraction method was not completely successful raising the potential of biased results. Moreover, the DNA extracted from each sample was not extracted from the same cerebral hemisphere. According to Telenius *et al*. (1994), the degree of CAG expansion increases in the cerebral cortex and basal ganglia regions of the brain. Nevertheless, this study included strong authority when it comes to the number of patients and samples, which were sufficient, and a wide range of age was included. Most importantly, blind-testing patients without knowing their family history in order to avoid false judgmental results when working with neuropathological examination in order to predict the accuracy. It was confirmed that the physiological and behavioral alteration differs from early to late stages of Huntington's disease. According to Raymond *et al*. (2011), genetic therapy must target patients coinciding to the stage of the disease.

CONCLUSION

There is a significant inverse correlation between age onset and number of CAG repeats which was confirmed in all studies but the severity of the disease in relation with the number of CAG repeats varied. This is understandable since the symptoms of the disease are diverse and every study linked the progression of illness with one or more of relative symptoms whether related to neurodegeneration or behavioral symptoms. Still, CAG repeat length has been reported to have subtle effect on rate of progression. Minor effects are clinically important to consider over time since HD is a complex disease. These outcomes of the CAG length may be relevant in the analysis of clinical trials. Other genetic mutations and environmental factors play an important role in the progression and neurodegeneration of Huntington's disease. (vanDellen and Hannan, 2004) These findings may have fundamental value and are crucial for the design and interpretation for future therapeutic trials.

References

1. Bates, G. P. (2005) History of genetic disease: The molecular genetics of Huntington disease — a history. *Nature Reviews Genetics*6(10), pp.766-773.
2. Brandt, J., Bylsma, F. W., Gross, R., Stine, O. C., Ranen, N. and Ross, C. A. (1996). Trinucleotide repeat length and clinical progression in Huntington's disease. *Neurology*, 46(2), pp.527-531.
3. Cattaneo E, Zuccato C, Tartari M (2005). Normal huntingtin function: an alternative approach to Huntington's disease. *Nat. Rev. Neurosci.***6**, pp.919–30.
4. Downing, N. R., Kim, J.-I., Williams, J. K., Long, J. D., Mills, J. A., Paulsen, J. S., & The PREDICT-HD Investigators and Coordinators of the Huntington Study Group. (2014). WHODAS 2.0 in prodromal Huntington disease: measures of functioning in neuropsychiatric disease. *European Journal of Human Genetics*, 22(8), pp.958–963.
5. Foroud, T., Gray, J., Ivashina, J., &Conneally, P. (1999). Differences in duration of Huntington's disease based on age at onset. *Journal of Neurology, Neurosurgery, and Psychiatry*, *66*(1), pp.52–56.
6. Ha, A. D., Beck, C. A., & Ha, J. (2012). Intermediate CAG Repeats in Huntington's Disease: Analysis of COHORT. *Tremor and Other Hyperkinetic Movements (NY)*, 2, tre–02–64–287–4.
7. Kieburtz, K., MacDonald, M., Shih, C., Feigin, A., Steinberg, K., Bordwell, K., Zimmerman, C., Srinidhi, J., Sotack, J., Gusella, J., *et al*. (1994). Trinucleotide repeat length and progression of illness in Huntington's disease. *Journal of Medical Genetics*, 31(11), pp.872-874.
8. Morrison, P. (2013). Huntington disease in 2013 - genetic choices across the life cycle. *Clinical Genetics*, 85(1), pp.76-77.
9. Raymond, L. A., André, V. M., Cepeda, C., Gladding, C. M., Milnerwood, A. J., & Levine, M. S. (2011). Pathophysiology of Huntington's Disease: Time-Dependent Alterations in Synaptic and Receptor Function. *Neuroscience*, *198*, pp.252–273.
10. Rosas, H. D., Reuter, M., Doros, G., Lee, S. Y., Triggs, T., Malarick, K., Hersch, S. M. (2011). A Tale of Two Factors: What determines rate of progression in Huntington Disease? A Longitudinal MRI study. *Movement Disorders*, 1691–1697.
11. Telenius, H., Kremer, B., Goldberg, Y., Theilmann, J., Andrew, S., Zeisler, J., Adam, S., Greenberg, C., Ives, E., Clarke, L. and Hayden, M. (1994). Somatic and gonadal mosaicism of the Huntington disease gene CAG repeat in brain and sperm. *Nat Genet*, 6(4), pp.409-414.
12. vanDellen, A. and Hannan, A.J. (2004) Genetic and environmental factors in the pathogenesis of Huntington's disease. *Neurogenetics* 5, pp.9–17.
13. Xuereb, J., MacMillan, J., Snell, R., Davies, P. and Harper, P. (1996). Neuropathological diagnosis and CAG repeat expansion in Huntington's disease. *Journal of Neurology, Neurosurgery & Psychiatry*, 60(1), pp.78-81.

How to cite this article:
Al-Kamali, F *et al*., Cag Expansion Length Correlation With The Rate Of Clinical Progression In Huntington's Disease. *International Journal of Recent Scientific Research Vol. 6, Issue, 7, pp.5175-5179, July, 2015*

****