



# Understanding the association between Mitochondrial DNA copy number and Telomere length in Huntington's disease patients.



Sowmya Devatha Venkatesh<sup>1</sup>, Pradip Paul<sup>1</sup>, Biju Viswanath<sup>1</sup>, Ravi Yadav<sup>2</sup>, Mathew Varghese<sup>1</sup>, Sanjeev Jain<sup>1</sup>, Meera Purushottam<sup>1</sup>.

1. Department of Psychiatry, 2. Department of Neurology, National Institute of Mental Health and NeuroSciences, Bengaluru, India.

## Aim:

To compare the relative telomere length (RTL) and relative mitochondrial DNA (mtDNA) copy number between HD patients and controls. To look for possible correlations between the two and check for the effect of DNA maintenance pathway and telomere associated SNPs. To evaluate the influence of these genetic factors on age of onset of HD.

## Background:

Mitochondrial and telomere function is shown to be compromised in Huntington's disease (HD). Various studies have shown a reduction in mitochondrial DNA copy number and telomere attrition in HD. The residual age of onset has been shown to be affected by genetic modifiers. DNA maintenance pathway genes such as Fan1, PMS2, MLH1, MSH3, LIG1 are shown to be strongly associated with HD pathology.

## Methodology:

- HD patients (N = 159) from the clinical services of NIMHANS and healthy controls (N = 127) were studied after taking informed consent. Ethical clearance was taken from the institute for the study.
- Relative mtDNA copy number and RTL was assessed along with single copy gene using qPCR with specific primers.
- $2^{-\Delta\Delta C_t}$  was calculated for each experiment as the measure of relative mtDNA copy number and RTL.
- The genetic modifier SNPs associated with DNA maintenance pathway (DMP) (rs3512, rs3791767, rs7936234, rs3730945, rs175080, rs1805323 and rs1799977) (1,2) and telomere associated SNP -rs12696304 were genotyped by Fluidigm genotyping technique.
- Mann Whitney - U test and Spearman rank correlation was used to study the relative mtDNA copy number and RTL in HD patients and controls.
- Linear regression has been used to study the effect of SNPs on RTL and relative mtDNA copy number with gender as covariate.

## Table 1: Demographic details:

	HD	Controls
<b>Total subjects</b>	159	127
<b>Male : Female</b>	102 : 57	59 : 68
<b>Age range years</b>	25-71	20 - 82
<b>Age (mean ± SD) years</b>	45.25 ± 10.92	39.34 ± 15.96
<b>DOI range years</b>	0.5 - 21	-
<b>DOI (mean ± SD) years</b>	5.25 ± 4.03	-
<b>CAG repeats Range Higher allele</b>	39-73	18-29
<b>CAG repeats Higher allele (mean ± SD)</b>	44.37 ± 4.03	19.1 ± 2.99

## References:

1. Lee, et al *Cell* 178 (4): 887-900.e14. <https://doi.org/10.1016/j.cell.2019.06.036>.
2. Bettencourt, et al." *Annals of Neurology* 79 (6): 983-90. <https://doi.org/10.1002/ana.24656>.

## Correspondence:

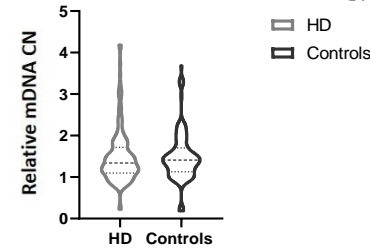
Sowmya D.V, PhD student  
Molecular Genetics Lab, Department of Psychiatry  
National Institute of Mental Health and Neurosciences  
**email:** sowmyadv@gmail.com

## Acknowledgement:

The research project is funded by SERB-DST- New Delhi.  
National Institute of Mental Health and Neurosciences.

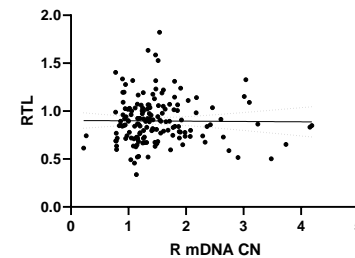
## Results

**Fig1: Relative measure of mitochondrial DNA copy number**



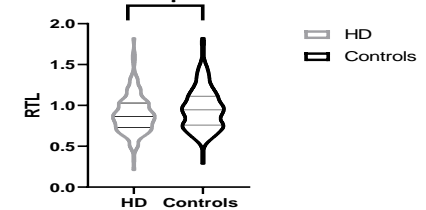
There is no significant difference of relative ratio of mitochondrial DNA copy number between HD - Median = 1.34 and controls - Median = 1.41, p value = 0.368

**Fig3: Correlation plot between relative mitochondrial DNA copy number and relative telomere length in HD**



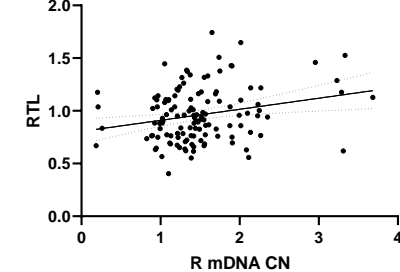
No significant association between CAG repeat length and relative telomere length,  $r = 0.0577$ ,  $p$  value = 0.469.  $N = 159$

**Fig2: Relative measure of telomere length**



Relative telomere length (RTL) is significantly lower in HD (Median = 0.866) compared to controls (Median = 0.946),  $p$  value = 0.0357. (Mann - whitney U test).

**Fig4: Correlation plot between relative mitochondrial DNA copy number and relative telomere length in controls**



Significant association between CAG repeat length and relative telomere length,  $r = 0.197$ ,  $p$  value = 0.025.  $N = 127$

- ❖ Relative telomere length (RTL) showed significant decrease in HD cases in comparison to controls.
- ❖ Relative mitochondrial copy (mtDNA) number did not show significant difference between HD case and controls.
- ❖ RTL significantly showed positive association with relative mtDNA copy number in controls but not in HD cases.
- ❖ The DMP pathway SNPs did not show any significant effect on RTL and relative mtDNA copy number with individual SNP score and also with global score - RTL ( $p = 0.79$ ) and relative mtDNA copy number ( $p = 0.316$ ).
- ❖ The DMP pathway SNPs did not show an effect on residual AAO with individual SNP score or the global score ( $p = 0.864$ ).
- ❖ Telomere associated SNP did not show significant effect on RTL ( $p = 0.702$ ).

## Discussion:

Telomere attenuation was observed in HD. Although previous studies showed a significant reduction in relative mtDNA copy number, we did not observe any significant reduction in HD compared to controls. DNA maintenance pathway SNPs and telomere-associated SNP did not show any effect on RTL, relative mtDNA copy number, and residual AAO. As the study is underpowered, further experiments are being undertaken to increase the sample size.