Cross-Sectional Gender Comparison of Gray Matter Concentration in Prodromal Huntington’s Disease

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Huntington’s disease (HD) is a neurodegenerative disorder caused by an abnormal repetition of the cytosine-adenine-guanine (CAG) trinucleotide in the \textit{HTT} gene. This polyglutamine mutation causes behavioral, cognitive, and motor abnormalities such as chorea. Additionally, HD is characterized by striatal atrophy prior to symptom onset. This experiment investigated gender contributions in pre-diagnosed or prodromal HD stages as well as striatal effect on motor dysfunction. All prodromal HD participants were positive for the genetic mutation. This cross-sectional study correlated striatal volume between men and women at different stages of prodromal HD. It was expected to see greater gender differences with increasing prodromal HD stages due to past HD gender studies. Structural Magnetic Resonance (sMRI) data were collected from more than a thousand cans and decomposed into specific regions via independent component analysis. Regions within the striatum became gray matter profiles of interest. Statistical analyses were conducted to quantify a relationship between gender and stage as well as striatal effect on motor dysfunction. Gender did not correlate with striatal volume at different prodromal stages yet striatal volume correlated with motor abnormalities as expected. Additionally, striatal volume decreased with increasing prHD stages. The results supported previous findings associating striatal volume and prHD stages, yet gender contributions were not identified. A study limitation is the use of a cross-sectional analysis; interactions between gender and rate of striatal atrophy have yet to be studied longitudinally in a prodromal HD cohort. Indexing prodromal progression factors could enhance therapeutic targets by isolating the earliest-affected regions of the brain in both sexes, which is vital for future treatment.

Abbreviations: HD – Huntington’s disease; prHD – prodromal HD; GM – grey matter; MANCOVA – multivariate analysis of variances

Keywords: Gray matter, Prodromal Huntington’s Disease, Magnetic Resonance Imaging, Gender, HD stage, Component

Introduction

Huntington’s disease (HD) is a genetic neurodegenerative illness caused by an abnormal expansion of the cytosine-adenine-guanine (CAG) trinucleotide repeats on the \textit{huntingtin} (\textit{HTT}) gene in chromosome 4 (McColgan and Tabrizi, 2018). The mutation is fully penetrant at 40 or more CAG sequence repeats in the \textit{HTT} gene (Wyant et al., 2017). The CAG sequence inversely correlates with disease onset and explains approximately sixty percent of variability in age of onset (Biglan, 2013; Fazio et al., 2018). HD clinical onset is characterized by extrapyramidal movement disorders such as chorea, dystonia, bradykinesia, rigidity, or oculomotor dysfunction is present (Paulsen et al., 2014b). The disorder leads to life dependency due to inhibition of cognitive, motor, and behavioral functioning (Ross et al., 2014). There is no current cure, and the usual
period from onset to death is fifteen to twenty years (Finkbeiner, 2011).

Structural and functional changes in the brain as well as subtle cognitive, behavioral, and motor changes begin years before onset (Snowden, 2017). Individuals who are genetically HD positive may exhibit signs of HD; they are characterized as pre-diagnosed or prodromal HD (prHD) (Misiura et al., 2017). Individuals with prHD have helped identify cognitive, behavioral, and biological markers in progression since said individuals have reduced cognitive performance, lower motor scores, and decreased gray matter (GM) volume (Roos, 2010). PREDICT-HD, a multisite prodromal study, found reduced GM concentration in the striatum (specifically the caudate and putamen) correlates with motor dysfunction in prodromal HD individuals (Paulsen et al., 2008; Coppen et al., 2018). In fact, striatal atrophy begins fifteen to twenty years prior to disease onset (Paulsen et al., 2008). Thus, striatal volume is a novel HD biomarker for HD progression. Additionally, PREDICT-HD utilizes the CAG Age Product (CAP) formula to index disease stage in prHD individuals. The CAP formula is age multiplied by CAG repeats minus a constant (CAP= age* (CAG-C)). Based on the equation, prHD individuals are grouped as low, medium, or high disease load based on smaller to larger CAP scores (Zhang et al., 2011). The equation is beneficial for categorization yet prHD gender effects on striatal volume are largely unknown.

Gender differences in HD is not as understood compared to common neurodegenerative disorders such as Parkinson’s disease (PD) and Alzheimer’s disease (AD). However, clinical markers vary between genders in PD and AD. A PD gender study found diagnosed women had more dopamine transporter in the striatum (putamen and caudate) than diagnosed men; the reduced amount of dopamine in men could contribute to faster PD onset (Lee et al., 2015). Additionally, an AD study revealed women had greater hippocampal volume than men and performed better on immediate and delayed verbal memory tests.

HD gender studies have shown variation in brain structure and motor symptom during development and post-diagnosis. A study found CAG repeat sequence length, below disease threshold, influenced brain structure in children at-risk for HD; on average girls had greater CAG expansion which lead to larger putamen and thalamus size while boys had smaller putamen density (Lee et al., 2017). An HD-diagnosed gender study assessed disease severity in motor, functional, cognition and behavioral symptoms at the first visit. Women showed a faster rate of progression than men in functional and motor domains. Women diagnosed with HD had significantly greater motor symptom severity than diagnosed men (Zielonka et al., 2018). However, a comparison of striatal volume between diagnosed individuals is unknown. Overall, these studies suggest girls and women with HD positive mutations are more susceptible to changes during development and HD diagnosis. It is requisite to understand how the brain changes during the prodromal phase. A prHD gender comparison of striatal volume has not been done.

In this study, the striatum of men and women in different prHD stages (control, low, medium, and high) and assess striatal effect on participant motor dysfunction were compared. The caudate nucleus and putamen are analyzed as GM concentrations. Based on past HD gender studies, the results should yield an association between prHD women and increasing prHD stages. As expected from past literature, the high prHD stage will have the least striatal volume compared to earlier prHD stages because striatal volume will decrease with proximity to onset.

Material and Methods

Participants

All imaging data for this analysis was obtained through the PREDICT-HD database. A total of 3700 scans (2825 prodromal and 875 control) were collected yet only scans from the first visit (N=1337) were used to maximize the number of available scans (see Table 1). Genotyping was performed on every participant prior to enrollment. All PREDICT-HD prodromal subjects are at-risk for HD (having a parent with HD) and were positive for the
mutated HTT gene from elective pre-symptomatic genetic testing (Klöppel et al., 2009). Thus, prHD individuals had more than 36 CAG repeats compared to healthy controls who had less than 36 CAG repeats in the HTT gene. The CAP formula (Zhang et al., 2011) was used to categorize participants into prHD stages as seen in Table 2. Finally, every participant provided informed consent and were treated with appropriate protocols approved by each participating institution’s internal review board; detailed enrollment criteria can be found in previous studies. Participants with other central nervous system conditions or unstable medical or psychiatric disorders were excluded from the study.

<table>
<thead>
<tr>
<th>Controls</th>
<th>Low</th>
<th>Mid</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/Male</td>
<td>18/19</td>
<td>16/18</td>
<td>23/24</td>
</tr>
<tr>
<td>Mean age</td>
<td>44.45</td>
<td>33.82</td>
<td>46.64</td>
</tr>
<tr>
<td>Mean CAG repeat</td>
<td>20.28</td>
<td>19.06</td>
<td>22.26</td>
</tr>
<tr>
<td>Mean SPM</td>
<td>(±3.05)</td>
<td>(±1.38)</td>
<td>(±5.27)</td>
</tr>
</tbody>
</table>

Imaging Data Processing
Imaging data were attained from 97 different scanners with a scanner field strength of 1.5 T or 3 T. Known influences of site scanner on GM were excluded using a linear regression model on each GM voxel. Scans were analyzed using SPM8 in MATLAB. T1-weighted MRI scans were segmented, unmodulated (to isolate GM concentration) and normalized using the default SPM8 pipeline, as the optimized pipeline (Good et al., 2001). Next, normalized data was smoothed to a full-width-half-maximum (FWHM) Gaussian kernel of 10 mm with a SPM8 software package; processed images were 90*109*91 voxels in size.

Source-Based Morphometry
Next, Independent Component Analysis (ICA) was applied to the preprocessed data using Source-based morphometry (SBM). ICA is a statistical and computational technique that decomposes mixed signals into maximally independent components. Different brain regions covary with each component/network grouping voxels among subjects to make up ICA (Xu et al., 2009).

Ninety-five GM components were estimated from the first visit scans. Using the infomax algorithm for spatial ICA, this gray matter by subject matrix was decomposed into a mixing matrix, representing the relationship between 1337 participants and 95 components, and a mixed matrix, representing the relationship between the 23 components and brain voxels. Participants’ loading coefficients describes how strongly the component manifest in the participants’ imaging data (Zhang et al., 2018). All 95 components were stable with 93 having minimum stabilities above 0.95 and only 2 had minimum stabilities below 0.90 (components 44 and 45 had stabilities of 0.85 and 0.72, respectively). Brain regions in each component were viewed via Xjview. From there, three gray matter components were selected from the 95 components: large putamen, small putamen and caudate. The components of interest are shown in Figure 1 below and location of the maximal region is displayed in Table 2.
**Statistical Analysis**

A cross-sectional Multivariate Analysis of Variances (MANCOVA) was conducted in R using the jmv package version 1.2.5 (Selker et al., 2020). The MANCOVA used three different variables to investigate a possible interaction between gender and CAP group within specific GM regions. The full code is available in the R script in Appendix A. This assessment was followed by a one-way analysis of variance (ANOVA) to identify the relationship between gender and prHD stage in component A which is listed in Appendix B. Next, the ANOVA quantified the relationship between prHD stages and motor score. Motor dysfunction is a hallmark of HD. The Unified Huntington’s Disease Rating Scale Total Motor Score (UHDRS-TMS) assess various domains of motor disability in HD. Thus, a motor score is indicative of enhanced motor disability (Reilmann and Schubert, 2017). The R script for the analysis as well as instructions for the ANOVA are listed in Appendix C.

The three dependent variables used in the MANCOVA were the three GM components of interest (see Table 2). Loading coefficients from each participant’s GM profiles were compiled into R. The independent variables were gender and stage (low, medium, high), while the dependent variable was the GM coefficients.

**Results**

Table 1 indicates the demographics of participants at the first visit.

**MANCOVA: Effects of stage**

Table 3 shows the results of the MANCOVA investigating gender (A) and prHD stages (B) and their interaction, in all three GM profiles combined. The MANCOVA did not produce an interaction between prHD stages and gender the cumulative GM profiles, yet there was a main effect of prHD stage in the cumulative component volume. (F(3,1)=4.91, p<0.001).

**ANOVA: No effects of gender**

Table 4 shows the results of the ANOVA investigating an interaction between gender (A) and prHD stages (B) in component A (large putamen). Results reveal a main effect of prHD stages in the large putamen volume (F(1,3)=14.34, p<0.001). Component A volume is decreasing with increasing prHD stages. There was no interaction between gender and stage or significant main effect of stage in component B (F(3,1)=1.815, p=0.142) or component C (F(3,1)=1.951, p=0.119).

Figure 3 is a visual representation of Table 4. The graph compares the striatal volume in component A between prHD stages.

Figure 4 displays the component volume between genders in prHD stages. Men and women experience equal striatal atrophy at
increasing stages of prHD. The graph depicts a positive correlation because the graph was multiplied by negative one.

**ANOVA: Effects on motor score**

Gender (A) does not interact with prHD stages to alter motor score. In Table 5, the ANOVA shows a main effect of prHD stage (B) (F(3,1)=111.42, p<0.001). The prHD stages (B) does influence motor score within the sample. Figure 6 is a box and whiskers which shows a relationship between prHD stages and motor score. There is a positive correlation between prHD stages and motor score. Thus, prHD participants have enhanced motor severity with increasing prHD stage.

![Graph showing component volume between genders in prodromal HD stages](image)

**Discussion**

Striatal volume reductions are evident throughout prHD phases (Paulsen et al., 2014b), yet gender contribution to the decline is largely unknown. Thus, we sought to identify an interaction between gender and prHD stages in striatal volume as well as how prHD stage effects motor scores, while covarying for age and scanner. The results did not support some of our hypothesis. Gender did not contribute to striatal volume at any prHD phase. Additionally, women in the high prHD stage did not have higher motor severity although a previous study showed women had higher motor severity compared to men (Zielonka et al., 2018). However, there were main effects of stage in the statistical analysis which did support previous work.

The first MANCOVA showed a main effect of stage in a cumulative combination of components. The MANCOVA revealed a significant effect of prHD stage in the large putamen (component A). The volume in the large putamen decreased with increasing prHD stage which is supported by PREDICT-HD findings (Paulsen et al., 2008). Subsequent loss of striatal neurons leads to severe atrophy, which is associated with prominent motor symptoms, an indication of HD onset (Reiner and Deng, 2018). Additionally, post-mortem studies reveal massive striatal atrophy in HD patients (Ghosh and Tabrizi, 2018). However, the significance of the putamen compared to the caudate is peculiar in this study. Past literature
found caudate neurodegeneration was more correlated with disease severity compared to the putamen (D et al., 2016). Regardless, the MANCOVA suggests both genders are equally subject to striatal atrophy prior to HD onset.

The ANOVA indicated a main effect of prHD stage on motor score, which means all prHD participants in the high group experienced the most abnormal motor movement compared to lower prHD stages. As stated earlier, abnormal motor movement is indicative of HD onset (Paulsen et al., 2014a) and motor severity is positively correlated with increasing prHD stages. The ANOVA result suggests prHD participants in the high group are closer to HD onset than prHD individuals in the lower stage. However, this analysis contained certain limitations.

A possible limitation in this study could be assessing gender and brain volume interactions for only the first visit participants. Although this analysis included a larger sample size, it was limited to one visit. Therefore, a possible future direction could be to investigate a longitudinal interaction between prHD genders and striatal volume. Striatal volume atrophy is evident prior to onset for both genders (Paulsen et al., 2008). Thus, it would be interesting to see if gender contributes to atrophy over time.

Another limitation could have been the choice to focus only on the striatum. The striatal region undergoes the most dramatic change during the prHD stages yet other brain regions depict atrophy. We found various regions deteriorate in prHD phases: frontal regions lost GM in the low prHD stage; temporal regions lost GM in the late prodromal HD stage; while parietal and occipital areas had co-occurring GM reductions (Ciarochi et al., 2016). Thus, future studies could compare atrophy in multiple brain regions between prHD men and women.

Furthermore, analysis of different brain regions could unravel an interaction between prHD men and women at distinct prodromal stages. There is growing evidence that gender influences etiology, appearance, and treatment outcomes of various diseases. However, gender contributions to neurodegenerative disorders is scanty especially in HD studies. There are different areas to expand on this project; from consistent scan collection overtime to investigating more brain regions that undergo atrophy. Gender contributions in Huntington’s Disease could pave the way for advanced therapeutics and serve as a precursor treatment for more common disorders (i.e. Parkinson’s, Alzheimer’s).

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References


Appendix A: Cumulative MANCOVA Rscript

```r
#install library
library(jmv)
#set working directory to location of csv file then view it
setwd("C:/Users/dogbul/OneDrive - Georgia State University/Research Project Data/MANCOVA")
read.csv("~/Research Thesis/MANCOVABASELINE.csv")
#label my variables
c1=MANCOVABASELINE$Comp.1
c2=MANCOVABASELINE$Comp.4
c3=MANCOVABASELINE$Comp.6
A=gender
B=stage
#Y is all my dependent variables
Y= cbind(c1,c2,c3)
#Now solve for mancova of Components
fit1=manova(Y~A+B+A:B)
#make sure to view MANCOVA
summary(fit1)
```

Appendix B: Component A ANOVA Rscript

```r
#install library
library(jmv)
#set working directory to location of csv file then view it
setwd("C:/Users/dogbul/OneDrive - Georgia State University/Research Project Data/MANCOVA")
read.csv("~/DO_HD/DO Stats/HD_primarydata.csv")
View(HD_primarydata)
#label all my variables
A=HD_primarydata$gender
B=HD_primarydata$stage
Y=HD_primarydata$Comp.1
fits=manova(Y~A+B+A:B)
summary.aov(fits)
#main effect of stage in Component A
```

Appendix C: Motor Score ANOVA Rscript

```r
#install library
library(jmv)
#set working directory to location of csv file then view it
setwd("C:/Users/dogbul/OneDrive - Georgia State University/Research Project Data/MANCOVA/UHRDBaseline.csv")
read.csv("UHRDBaseline.csv")
#label my variables
Y=UHRDBaseline$UHDR
A=UHRDBaseline$gender
B=UHRDBaseline$X.Stage
#Y is all my dependent variable (tms)
#Now solve for ANOVA of total motor score
fits= aov(Y~A+B+A:B)
summary.aov(fits)
#main effect of stage on motor score
```