**PREVALENCE OF INFLAMMATORY DISORDERS IN HUNTINGTON’S DISEASE**

by

**Michaela Anne Holliday**

BS Biological Sciences, BS Microbiology

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**ABSTRACT**

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This essay is submitted

by

**Michaela A. Holliday**

on

April 19, 2018

and approved by

Essay Advisor:

Candace M. Kammerer, BS, PhD \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Program Director, MPH in Public Health Genetics

Associate Professor, Human Genetics

Graduate School of Public Health

University of Pittsburgh

Essay Reader:

Allison L. Kuipers, BS, PhD \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Assistant Professor, Epidemiology

Graduate School of Public Health

University of Pittsburgh

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Candace M. Kammerer, BS, PhD

**PREVALENCE OF INFLAMMATORY DISORDERS IN HUNTINGTON’S DISEASE**

Michaela A. Holliday, MPH

University of Pittsburgh, 2018

**Background:** Huntington’s disease (HD) is a devastating, neurological, genetic disease characterized by the degradation of neural cells over time. No treatment is currently available and long-term care is costly. Inflammatory disorders, such as Multiple Sclerosis (MS) and arthritis, are characterized by an overactive immune system. The relationship between inflammation and neurodegeneration is poorly understood, but studies indicate that alleles associated with immune response and neuroinflammation are enriched in HD patients. Using data from a multinational, observational study (Enroll-HD), I assessed whether the presence of two inflammatory disorders, MS and arthritis, was higher among individuals with manifest HD compared to those who had either pre-manifest HD, were genotype negative or were family controls. This study is important for public health because HD is understudied, resulting in a lack of knowledge regarding comorbidities, possible treatments, and appropriate care.

**Methods:** Data were available on 8,714 Enroll-HD participants (4,752 manifest, 1,862 pre-manifest, 1,089 genotype negative and 1,011 family controls) with a mean age of 49 years (range 18-91 years) Over 87% of the cohort self-identified as Caucasian; therefore, subsequent analyses were done in Caucasians alone (n=8,089).

**Results:** Overall, 4% had arthritis, 0.3% had MS, 46% drank alcohol, and 25% smoked. Manifest-HD participants were less likely to drink compared to pre-manifest, genotype negative and family controls (38%, 60%, 53%, and 54% respectively, p<0.001) and more likely to smoke (27%, 26% 20% and 18%, respectively, p<0.001). The prevalence of MS was lower among individuals with manifest HD than among pre-manifest and genotype negative individuals (0.2%, 0.4%, and 0.42% respectively). The prevalence of arthritis was similar among manifest HD, pre-manifest and genotype negative individuals, but not for family controls (~4% versus 7.4%, respectively).

**Conclusions:** In this study, individuals with manifest HD were less likely to drink alcohol and more likely to smoke than individuals in the other two groups, which is consistent with previous studies. However, the prevalence of MS or arthritis was not higher among those with manifest HD. Additional studies using serum markers of inflammation need to be performed to assess the potential associations of HD prognosis and of neuroinflammation.

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preface

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# huntington’s DiseasE (HD)

Huntington’s disease (HD) is a progressive, neurological, autosomal dominant genetic disorder [1-3]. It is characterized by three different groups of symptoms: movement disorders, behavioral and/or psychiatric disorders, and cognitive disorders [1, 2]. Usually, the onset of one group of symptoms (i.e. movement, behavioral/psychiatric, cognitive) is related to another; all three groups are related to HD neuropathology [1]. Approximately two-thirds of HD patients manifest neurological symptoms as initial signs and symptoms of the disease, while the other one-third manifest psychiatric symptoms first [4]. The symptoms advance with the overall progression of this chronic illness [5].

Although the symptoms of HD vary between affected individuals, several common symptoms are observed in HD patients [1]. Movement disorders include both those that are involuntary and those that are voluntary [4, 6, 7]. Impairments that result from problems with voluntary movements are more closely associated with an individual’s day-to-day activities, as well as the preservation of their independence [6]. The increase in voluntary movements is correlated with an increase in cognitive impairment, which is typical in HD [7]. A common symptom attributed to involuntary movement is abnormal, uncontrolled, involuntary and random movements, known as chorea [1, 2, 5, 8]. Chorea is a major sign of HD, and manifested in more than 90% of patients [4]. Also, HD patients have difficulty walking; their balance and posture are impaired because they are dystonic [1, 6], and they have involuntary muscle contractions [9] or are rigid [6]. HD patients also have difficulty swallowing and speaking, and their speech may be slurred [1, 8]. Several psychological disorders are associated with HD and its progression. The most common disorder is depression [6], but other disorders include heightened irritability, personality changes [1, 10], obsessive-compulsive behavior, mania and bipolar disorder [6]. Patients with HD have impaired cognition, which results in difficulty remembering things, lack of impulse control, and lack of awareness [1, 2, 6]. In general, the worsening of symptoms (motor, behavioral/psychiatric, and cognitive impairment) depends on disease stage [7].

HD may be diagnosed based on the presence of motors symptoms, such as chorea. This diagnosis may be confirmed with genetic testing if needed or desired [11]. The Unified Huntington’s Disease Rating Scale (UHDRS) was developed for use in a clinical setting to assess four domains: motor, cognitive, behavior, and functional capacity [12]. This scale is used to assign patients to different diagnostic categories associated with HD, by assigning them a Diagnostic Confidence Level (DCL) [4]. This scale is also useful to track changes in the clinical features of HD over time [12]. Table 1 shows the categories of HD, whether genetically confirmed or not, and what DCL physicians would most likely assign to each patient [13, 14]. Once an individual is diagnosed with HD, the time to death is likely between 10 and 30 years [6]. Individuals with juvenile HD, characterized by an earlier age of onset and faster disease progression [8], typically die within 10 years after diagnosis [6].

Table 1. Diagnostic Confidence Level assigned by certified motor rater as to whether an individual “meets the operational definition” for a “movement disorder in a subject at risk for HD”.

|  |  |
| --- | --- |
| **DCL** | **Definition of impairment** |
| Motor DCL= 0 | Unimpaired, normal (no abnormalities) |
| Motor DCL= 1 | Non-specific motor impairments, less than 50% confidence  Onset of motor impairments |
| Motor DCL= 2 | Motor abnormalities may be signs of HD (50-89% confidence) |
| Motor DCL= 3 | Motor abnormalities that are likely signs of HD (90-95%) |
| Motor DCL= 4 | Motor impairments clear signs of HD (>99% confidence)  Onset of HD diagnosis |

Based on two studies, the overall costs associated with the burden of HD, especially in terms of stage of HD, are significant. Divinno et al. performed a study to assess the costs associated with healthcare in HD patients, as well as the factors that influence them. They used data on HD patients in the United States who were listed in commercial and Medicaid databases from 2002-2009. Medicare databases were not a part of this study. Patients were characterized by stage of HD and total costs per patient per stage of disease were measured to yield a total patient-year cost. The 1,272 HD patients from two healthcare sources (752 from the commercial database and 520 from the Medicaid database) had similar mean ages, 48.5 and 49.3 years respectively, but differed by stage of disease. In the commercial database, the proportion of individuals at different stages of HD, were similar, but most individuals in the Medicaid database were classified as manifest HD. This study reported that the mean total cost per year increased as the patient’s stage of HD increase. This increase was mainly attributed to healthcare costs such as in home care [15], indicating a lack of inpatient care facilities for this subset of patients. Jones et al. analyzed data on 131 HD patients, (72 women and 59 men), from the European Huntington’s Disease Network Registry (10% of people from the Registry) to assess the cost and potential burden of HD in the UK. The mean age overall was 50 years and the distribution of patients across each stage of HD was similar. Jones et al. reported that the calculated UK costs appear to be higher than those in the US. The researchers note that the healthcare systems in the UK and US differ, and these differences may contribute to the cost differences. Similar to results from Divinno et al., this study also reported that costs increase as disease severity increase. Furthermore, informal costs, such as those attributed to individuals providing in home care, were the largest contributor to total costs indicating a lack of adequate facilities to care for these patients [16].

Currently, no approved cure or treatment to slow the progression of the disease is known; however some of the disease symptoms may be managed [1]. Two approved drugs, tetrabenazine and deutetrabenazine, also known as Austedo [17], may help control involuntary movements associated with HD [18]. Austedo (2016) is only to be taken twice a day in contrast to tetrabenazine, which has to be taken three times a day [17]. This protocol is easier for HD patients and/or caretakers to be compliant. Although there is no approved cure for this disease, an ongoing clinical trial is being done to assess patient tolerance, safety, and long-term outcome of a newly developed drug called IONIS-HTTRX [18]. The drug is an antisense oligonucleotide that acts on the pre-mRNA for the huntingtin gene to decrease all huntingtin protein that is produced in the brain, and thus, potentially modify disease progression. Recently, the company that developed the drug, IONIS Pharmaceuticals, announced result of their clinical trial in which patients took the drug four times at four week intervals over the course of 13 weeks. The safety and tolerability of the drug is ongoing and will continue for a total of 29 weeks [18]. IONIS reported the drug was safe and well tolerated by patients and also lowered the level of both mutant and wild type huntingtin protein in the nervous system (measured via Cerebral Spinal Fluid, CSF). Furthermore, the trial results indicated an inverse dose-dependent relationship between the drug and the amount of huntingtin protein in the CSF [19].

The next steps will be to follow HD patients longitudinally to assess the drug’s effect on disease progression [20], and depending on the outcome, perhaps one day be able to state that a cure for HD exists. Currently, the probability of developing HD can be predicted using the CAP score. The CAP score is the probability of an individual being diagnosed with HD within a specific time frame, which takes into account an individual’s age and number of CAG repeats [21]. The CAP score assigned to an individual and explanations are described in Table 2 [21]. This variable is not included in the original dataset, it was calculated after the data was received.

Table . CAP score assigned and explanation.

|  |  |
| --- | --- |
| **CAP score** | **Explanation** |
| < 0 | Not likely or far away from a diagnosis, <50% chance of developing HD. |
| = 1 | 50/50 chance of developing in 5 years. |
| > 1 | >50% chance of developing in less than 5 years. |

In this essay, several abbreviations related to Huntington’s disease and inflammatory disorders will be used. They are provided in Table 3.

Table . Abbreviations used for the purpose of this MPH essay.

|  |  |
| --- | --- |
| **Abbreviation** | **Entire term** |
| HD | Huntington’s disease |
| *HTT* | Huntingtin gene |
| DCL | Diagnostic Confidence Level- assignment by a motor rater in regards to how confident they are that an individual has HD |
| UHDRS | Unified Huntington’s Disease Rating Scale |
| CAP score | Measure of chance of developing HD within 5 years |
| MS | Multiple Sclerosis |
| RA | Rheumatoid arthritis |
| PsA | Psoraratic arthritis |
| CIDs | Chronic inflammatory systemic diseases |
| HLA | Human leukocyte antigen |
| CNS | Central nervous system |
| TMS | Total Motor Score |
| TFC | Total Functional Capacity |
| PBA | Problem Behavior Assessment |
| HADS | Hospital Anxiety and Depression Scale |
| SIS | Snaith Irritability Scale |
| CSSR | Columbia Suicide Severity Rating Scale |

Additionally, four HD categories will be described and used for analysis in this essay. These include: (1) manifest HD, (2) pre-manifest HD, (3) genotype negative, and (4) family controls. Individuals with manifest HD test positive for the mutant *HTT* and are showing signs and symptoms of HD, whereas those with pre-manifest HD test positive for the mutant gene, but are not showing signs and symptoms at that point in the study. Genotype negative individuals are those who have a first-degree relative with HD, but do not have the mutant huntingtin gene. Lastly, family controls are individuals who do not have a first-degree relative with HD, but are still involved/affected by the disease. For example, such an individual might be the spouse of an individual with HD.

## Epidemiology of HD

Huntington’s disease is a single gene disorder, in that a mutation in the huntingtin gene (*HTT)* results in the manifestation of the disease [6, 8, 22]. The disease will appear in all individuals with a mutated *HTT* gene, but the number of the CAG repeats is the largest risk factor in terms of progression of HD and survival [23]. As the number of CAG repeats increases, the disease progresses faster and the length of survival decreases [4, 23-25]. Another risk factor for HD is familial aggregation; 90% of cases of HD are hereditary. Ten percent of individuals with HD do not have a family history of disease, but have a de-novo mutation in *HTT*. This de novo mutation arises because the number of CAG repeats that characterizes the disorder may increase during meiosis and result in offspring with HD [26]. In addition to the genetic features of HD, environmental risk factors also contribute to the prevalence of the disease including sex [27] and race [8, 28-34].

Incidence of HD varies by race and geography. Pringsheim and colleagues performed a review of the literature regarding the incidence and prevalence of HD; eight studies on incidence and 17 studies on prevalence were included. They performed a meta-analysis of incidence data from four of these studies. They reported that the overall incidence was 0.38 per 100,000 per year with a confidence interval of 0.16-0.94. In general, they observed a lower incidence in Asian populations(n=2) compared to those studies done in European, North-American and Australian populations (n=6) [28].

Although HD is observed in all populations, prevalence differs among races. Warby and colleagues report that prevalence of HD is highest (greater than five per 100,000) in Western European populations [31]. But according to the meta-analysis of 13 studies performed by Pringsheim et al., the worldwide prevalence of HD is 2.71 per 100,000. Eleven of these 13 studies were done in Europe, North America and Australia, and a meta-analysis of these 11 studies indicated that prevalence was 5.70 per 100,000 [28], which is similar to Warby and colleagues. The U.S National Library of Medicine states that the prevalence of HD among individuals with European ancestry is three to seven per 100,000 [28]. Finally, Harper used data from population surveys and death rates, and estimated that the prevalence of HD in most European populations was four to eight per 100,000 [29]. Therefore, the prevalence of HD in European populations varies among studies, but the general consensus is that the prevalence is higher among European populations and ranges from 3-8 per 100,000.

HD is not as common among other groups such as Japanese, Chinese or African descent [8]. Pringsheim et al. performed a meta-analysis on three prevalence studies done in Asia, and obtained an overall prevalence of 0.49 per 100,000 [28]. Warby et al. reported that the prevalence of HD in Eastern populations, such as China and Japan, was 0.1-0.5 per 100, 000. These estimates are approximately 10-100-fold lower than those from European populations, but this vast difference is not well understood [31]. Miao Xu and Zhi-Ying Wu reviewed 50 papers obtained from PubMed and China National Knowledge Infrastructure from 1994-2014 to better estimate the prevalence of HD in Asian countries. They reported that the prevalence of HD in Asians was 0.40/1000,000. They also determined from a recent epidemiological study in Taiwan (China) that the average annual incidence rate was 0.1/100,000 [32]. Due to a lack of research, the prevalence of HD in African populations is not well studied. In 1980, a national investigation was done to obtain better estimates of the prevalence of HD in South Africa; results of the study revealed that the prevalence of HD in this population was 0.1 per million [34]. Also, a study conducted in 1987 at Johns Hopkins University reported that the prevalence of HD among South African blacks to be 0.6 cases per million people [33]. Few follow-up studies on the prevalence of HD have been done in African populations.

In 2016, Baig et al. performed a systematic review in order to estimate the prevalence of HD following the establishment of diagnostic testing. They assessed data from 22 studies performed from 1993-2015 and concluded that the prevalence of HD was significantly lower in Asian populations compared to Western Europe, North American and Australia [30]. Thus, the results from current studies indicate that the prevalence of HD is highest among European populations, but the data is generally lacking with regards to the incidence and prevalence of HD in other racial groups. Because these populations are understudied, the incidence and prevalence of HD may be higher than is currently reported.

In addition to variation in incidence (or prevalence) across different ancestry groups, incidence differs by sex of the affected individual. Zielonka et al. performed a study involving 1,267 patients (50% women) with HD from the European Huntington’s Disease Network Registry to determine whether progression of disease differed by sex. A cross-sectional analysis, controlling for age of onset, disease burden, disease duration, smoking use, alcohol consumption, depression, and years of education, was done to assess the differences in various categories associated with HD: motor, functional and cognitive scores. Follow-up data was analyzed with linear mixed models to better understand possible sex effects on HD progression. Zielonka et al. found that women had a lower total functional capacity score (TFC) when compared to men (p<0.001) and higher UHDRS motor scores (p=0.033). Also, the results of the longitudinal analysis showed that disease progressed faster among women, as measured by their reduced functional capacity (p=0.025), reduced motor capacity (p=0.032) and reduced preservation of their independence (p=0.008). Thus, sex may influence the progression of HD, with women experiencing a faster progression over time, especially with regards to motor and functional ability [27].

## Genetics of HD

Huntington’s disease is an autosomal dominant disorder, meaning that both males and females are equally affected [1, 2, 35] although their disease progression may differ [27]. Each child of an individual who is affected by HD has a 50% chance of inheriting the allele that causes the disease [1, 5, 35]. The disease requires only one copy of the mutated gene in order for HD to occur [6, 8, 22]. Two copies of the normal huntingtin gene means an individual will not develop HD [35]. It only takes one mutant huntingtin gene to cause the disease; the disease outcome is not different just because a normal huntingtin gene is present [1, 35]. In some rare cases in which two mutant alleles are inherited, the child will develop HD and all of that child’s offspring will have HD [1, 35].

The disease is characterized by a repeat expansion of a trinucleotide codon, CAG, in exon one [36] of the huntingtin gene (*HTT*), on chromosome 4 [1] at band 4p16.3 [2, 37]. The CAG trinucleotide encodes the amino acid glutamine [22, 35]. This repeat expansion causes an abnormally long protein to form, resulting from this polyglutamine sequence [1, 3, 8, 22]. The number of times the CAG codon is repeated determines how many glutamine amino acids are included in the overall huntingtin protein. The number of CAG repeats ranges from less than 27 to greater than 40 [35]. The most common number of repeats in individuals ranges between 17-20 [22]. Figure 1 shows a general overview of how the number of CAG repeats determines the outcome of the huntingtin protein.



Figure . HD is characterized by a trinucleotide repeat expansion (CAG), in which a higher number of repeats results in an abnormal, mutant protein.

The number of CAG repeats in the huntingtin gene is positively correlated with an individual’s risk of developing HD (see Table 4) [2, 4], The age of onset of HD ranges from 3 years to 85 years [5]. The average age of onset is 40 years [5, 10]. Individuals who develop HD before age 20 have juvenile Huntington’s disease [6] and have a large number of repeats (more than 60) [8], faster disease progression [6] and account for 5-10% of HD cases [4]. Individuals who have fewer than 27 repeats have a normal phenotype [35] and do not develop HD. Individuals who have between 27-35 repeats have a normal phenotype, but the number of repeats may expand when transmitted to the next generation, due to the instability of the CAG repeat [4, 5, 22, 35]. Therefore, they are at risk of passing a mutant *HTT* gene to their children [8]. Repeats between 35-39 have a reduced penetrance, in other words, some individuals develop the disease and some do not [5, 8]. Lastly, those with 40 or more repeats will manifest the disease [8, 35, 37]. Table 4 lists the number of CAG repeat lengths and the interpretation in terms of disease manifest or lack thereof [35]. The number of CAG repeats corresponds to a genetic diagnosis of HD. The DCL assigned to an individual pertains only to manifest motor symptoms that may or may not be associated with HD.

Table . Relationship between number of CAG repeats and manifested HD.

|  |  |
| --- | --- |
| **CAG repeat length** | **Interpretation** |
| < 27 | Normal |
| 27-35 | Normal, mutable (sometimes called the “intermediate range”) |
| 35-39 | Abnormal, reduced penetrance (sometimes called the “indeterminate range”) |
| > 40 | Abnormal |

In addition to the positive relationship between number of CAG repeats and development of disease, the number of CAG repeats is inversely associated with age of onset of HD [22, 36]. In other words, the larger the expansion, the earlier the age of onset [4, 22, 35, 38], also the rate of decline is increased in motor, cognitive and functional symptoms [4]. However, increasing repeat number is not associated with specific symptoms (motor, cognitive, functional and/or behavioral), [25, 35] or with a decline in behavioral symptoms [4]. CAG repeats between 27 and 39 copies in length may expand in subsequent generations, a phenomenon called anticipation [8], especially if the mutant huntingtin gene is passed through the paternal line [4, 5, 22].

## Pathology of HD

The function of the huntingtin protein is unknown [39, 40], but in mouse models that lack the protein, the mouse is not viable [40]. Essentially, the knockout of the protein results in an increased death of cells in the body, and a disrupted transport of nutrients to the fetus. In mice that have two mutant *HTT* alleles, the knockout of one of the mutant alleles results in developmental defects [22, 39]. Zeitln et al. observed regionalized cell death –apoptosis- in the embryonic ectoderm layer of mice with two mutant alleles for *HTT*. These mice also express the two mutant *HTT* alleles in the ectoderm at a much higher level than normal; thus these researchers hypothesized that the normal huntingtin protein is involved in controlling cells from going through the apoptotic pathway [39]. In zebrafish the presence of the mutant protein also results in development defects. Adult zebrafish require functional huntingtin protein for the viability of their cells [22]. Thus it is hypothesized the huntingtin protein has an anti-apoptotic role in cells [22, 39], and is also required for normal embryonic development [22, 40].

The wild-type huntingtin protein has been proposed to be involved with several organelles in cells [3] as well as implicated in several intracellular processes in the body [41]. In terms of organelles, the protein is “associated with the plasma membrane, endocytic and autophagic vesicles, endosomal compartments, the endoplasmic reticulum, the Golgi apparatus, mitochondria and microtubules” [22]. Gil and Rego state that the normal huntingtin protein may have a role in many intracellular processes including “protein trafficking, vesicle transport, postsynaptic signaling, transcriptional regulation and apoptosis [41]”. However, the mechanism of action of the protein in these processes is unclear. Thus a loss of function of the normal *HTT* protein or likewise a gain of function mutant *HTT* protein leads to the disruption of both organelle and intracellular processes.

The mutant huntingtin gene, with an expanded polyglutamine sequence, confers a gain of function, which researchers hypothesize is toxic to the brain [22, 37]. However, the exact mechanism of the gain of function is still undetermined [1, 3]. Researchers further hypothesize that the elongated protein is processed differently in neurons, and that fragments of this protein accumulate over time to form intranuclear inclusions [4, 22, 35]. The fragments that are created are N-terminal huntingtin fragments that contain the expanded polyglutamine segment [4, 22] (the mutant protein). The fragments bind together and accumulate in the nucleus of neurons causing a disruption of specific transcriptional pathways in these cells [8, 22], leading to an overall breakdown of neuronal processes [22, 25] and over time, cellular degeneration [6]. Davies et al. observed that mice who were transgenic for exon 1 for the human *HTT,* that had between 115-156 CAG repeats, developed intranuclear inclusions in neurons; the neurons contained the mutant huntingtin protein. They also observed that these inclusions began to form before the development of any sort of neurological signs or symptoms [42]. Schilling et al. performed a study in which they created transgenic mice that expressed a cDNA encoding a N-terminal fragment huntingtin protein with either 82, 44 or 18 glutamines [43], which translates to 82, 44 or 18 CAG repeats. They observed that mice with 82 glutamines developed abnormalities such as loss of coordination, and died early. Mice displaying these functional effects and early mortality were also characterized by intranuclear inclusions. Based on these results the researchers concluded; (1) N-terminal mutant huntingtin fragments are harmful to neurons and (2), these fragments form intranuclear inclusions [43]. In later studies, Palfi and colleagues developed a non-human primate genetic model of HD. These researchers administered vectors that coded for a fragment of the mutant huntingtin protein, with 82 CAG repeats. Nine weeks after administration of the vectors, the researchers observed nuclear aggregates containing the mutant protein, which led to symptoms associated with HD such as spontaneous movement of the legs and arms [44]. These results provide further evidence that the mutant huntingtin protein causes N-terminal fragments to form that are detrimental to neurons, ultimately leading to the manifestation of symptoms associated with HD. Post-mortem studies regarding N-terminal fragments in adult HD post-mortem brains are limited [45]. A study by Mende-Mueller et al. looked at the breakdown of the mutant huntingtin protein in both the cortex and striatum of post-mortem samples from adult HD brains as well as control brains using Western blotting. They used “domain-specific anti-htt antibodies” that recognized N-terminal domains. Ultimately, they reported that the level of N-terminal fragments (40-50kDa) in the striatal of adult HD brains was higher than that of normal brain samples [46].

The overall pathology of HD can be characterized by a shrinkage of the brain [4, 10, 22]; which can be as much as 20% [47] and may be attributed to the degeneration of neurons in the caudate and putamen (corpus striatum) areas. Neuronal loss in these areas may be up to 90% in some patients at their time of death [10], indicating these areas of the brain may be most susceptible to the effects of the mutant huntingtin protein [22]. Additionally, the presence of the mutant huntingtin in the corpus striatum may be the main cause of neurodegeneration [36]. Rosas et al. performed a study in which they used MRI scans of 11 patients with HD and 13 age-matched subjects to better understand how HD affects the overall degeneration of the cortex. They were specifically interested in learning which regions are affected, how the degeneration develops over time, as well as the extent of the relationship between the degeneration and clinical symptoms of the disease. They observed an overall degradation of the cortex, which started early in disease progression, but the extent of degradation varied by the patient and stage of HD. Specifically, Rosas et al. found that the sensorimotor region of the cortex was most affected [48]; this result may indicate why motor symptoms in HD (such as chorea) are more commonly observed. These researchers propose that the variation in clinical symptoms of HD may be attributed to the variation observed in the degradation of the cortex depending on patient and stage of HD [48]. Results of another study by Rosas et al. indicate a possible mechanism for impairment in HD. The corpus callosum, a major band of nerves in the brain that join the two hemispheres, is involved in transferring sensory, motor and cognitive information between the two sides of the brain. The researchers examined the brains of controls, pre-manifest HD, and manifest HD individuals using “T1-weighted, diffusion tensor imaging and a modified corpus callosum segmentation.” Compared to the controls, individuals with pre-manifest and manifest HD had a loss of neurons and connectivity between the two hemispheres of the brain, providing evidence as to why there is a degradation of sensory, motor and cognitive processing in HD patients throughout their disease progression. The thickness of the corpus callosum was not significantly different between controls and pre-manifest HD. In contrast, the thickness of the corpus callosum was significantly smaller in each of the six region researchers measured when compared to controls [49].

## Inflammation and HD

HD is characterized by the degradation of neural cells, whereas inflammatory disorders are mainly attributed to an overactive immune system; a possible relationship between the two processes is unknown. For example, does an overactive immune system contribute to a degradation of neural cells or does the degradation of neural cells contribute to an overactive immune system? The relationship between inflammation and neurodegenerative diseases, such as HD, is still poorly understood [50]. The relationship between inflammation and neurodegenerative diseases, is termed neuroinflammation [37, 50], specifically the “activation of innate immune cells and expression of inflammatory molecules in the brain” [51]. It is unclear whether neuroinflammation is a result of the mutant huntingtin’s presence in the brain or if neuroinflammation has an effect on HD disease progression [36].

The activation of various immune cells (observed in the cerebral spinal fluid) has been correlated with the pathology of HD patients [50]. Of the cells related to the immune system, those most notable in the pathology of HD are described below. The presence of microglia [36, 37, 50, 51] that express proinflammatory cytokines [36, 51] has been detected. There is an increased presence of monocytes and T-cells, and an increase in anti-oxidant activity, which is part of the adaptive immune response. Also, the inhibition of astrocytes [50] has been observed, as well as the accumulation of complement factors in the striatum [36]. Lastly, macrophages appear to develop a defective migration pattern [36, 50, 51] in the brains of HD patients. Björkqvist et al. provide evidence that the innate immune system may be activated by the progression of HD. Based on analysis of 194 plasma samples from individuals who tested positive for the HD mutation, (in various stages of the disease) and controls, they suggested that the overall dysfunction of the immune system is related to the pathology of HD, specifically in the brain [52].

Neurodegeneration is a major symptom of HD; it involves the breakdown of neuronal processes [22, 25] and overall cellular degeneration [6]. Microglia that are activated in the brains of HD patients have been hypothesized to be involved in disease pathogenesis [53]. Typically, microglia cells are activated in response to adverse changes in the CNS, such as neurodegeneration, because they are involved in the first line of defense. Their function is to help repair tissue in the brain and regenerate neural cells [54]. In relation to HD, an increased presence of microglia has been found in pre-symptomatic HD gene carriers [36, 50], before the onset of symptoms [51], and may be detected up to fifteen years before the actual age of onset [50]. This suggests that active neurodegeneration and neuroinflammation may be occurring in the brains of HD patients before they present with disease symptoms. Also, the mutant huntingtin protein is expressed in microglia cells [36], further supporting the hypothesis that these immune cells are actively involved in HD. These observations may indicate that these cells have an active role in the progression of disease towards manifestation of symptoms [51]. Additionally, the activities of microglia are amplified by astrocytes. Normally, astrocytes function to help support the well-being of neurons, but the mutant huntingtin protein may create functional defect in the astrocytes that makes these immune cells more likely to support a pro-inflammatory state [50], and eventually leading to neurodegeneration and thus HD.

Another aspect of inflammation as it relates to HD involves the impaired recognition of macrophage receptors. This impairment is a result of mitochondrial dysfunction, caused by the presence of the mutant huntingtin protein in the brain. This dysfunction and loss of receptor recognition has been shown to cause an increase in an inflammatory response [36]. Additionally, complement factors may accumulate in the brain. They are expressed in the striatum, and may cause an overactivation of the complement system (part of the immune system) and lead to toxic effects in the brain such as neuronal degradation [36, 37].

Labadorf and colleagues describe how the role of genes and “transcriptional dysregulation” has been previously reported in cases of HD patients, but the processes underlying this potential role are not understood. These researchers used 20 HD samples and 49 controls frozen brain tissue samples from the prefrontal cortex to assess possible differences in mRNA expression using next generation high-throughput sequencing. They reported that genes related to immune response, neuroinflammation and development were enriched in HD patients compared to controls. Of the genes related to immune response, genes coding for cytokines receptors were implicated. Also, they found genes related to multiple immune system pathways were enriched in HD patients [10]. Based on these results, Labadorf and colleagues suggest that use of an anti-inflammatory therapy may help in treating HD [10] and potentially slowing down its progression [37].

In 2015, Vaccinex, Inc., a private immunotherapy company, partnered with the Huntington Study Group to investigate the safety of a new drug, VX15/2503, in those with early-manifest HD or manifest-HD [55]. This clinical trial, also known as SIGNAL, comprises two cohorts and is a “multi-center, randomized, double-blind, placebo-controlled study [that is] being conducted at approximately 30 sites across the United States and Canada”. The first cohort has been fully recruited and preliminary imaging data is available, the second cohort is currently enrolling participants.

VX15/2503 is a monoclonal antibody that is given through IV infusion. It targets the semaphoring 4D (SEMA4D) protein because this protein’s function is to help activate and move cells throughout the body, and may be involved in activating cells in the brain that lead to inflammation. The preliminary results from the first SIGNAL cohort show that this drug may show the progression of brain inflammation seen in HD, although the FDA has not yet approved it. Regardless, if the progression of HD is slowed, then clinical features of HD, such as impaired cognition, movement and behavior, may be reduced as well [56]. This ongoing clinical trial is using evidence-based research studies, such as those described above, to alter HD disease progression. This trial provides additional evidence that inflammation and an activated immune system may be a part of HD pathogenesis.

In summary, neuroinflammation is a strong component of HD pathogenesis. The mutant *HTT* alters normal immune cell processes, which leads to a chronic state of an inflammatory response, which leads to the death of neurons, which induces more inflammation, and finally, results in a feed forward loop of neuroinflammation [37, 50]. This cascade of events suggests that increased inflammation is a result of neuronal cell death that is caused by the mutant huntingtin protein [36, 37]. Alternatively, mutant huntingtin protein may directly cause increased inflammation [36].

# Inflammatory disorders

Inflammatory diseases, such as arthritis and Multiple Sclerosis (MS) are chronic inflammatory systemic diseases (CIDs) [57] that result in a debilitating disorder with an increased risk of mortality [57]. The prevalence of CIDs in the general population is 0.1-1% [58].

CIDs may result from (1) genetic variants that make an individual susceptible to the disease, (2) environmental priming, (3) continuous immune response against the body’s own cells and tissues, and (4) tissue destruction [59]. Several systems are involved with CIDs, including immune, nervous, endocrine and reproductive [58]. These diseases are characterized by the immune system being in constant overdrive as it is attacking the body, which results in massive energy expenditure [59].

Several genes have been associated with CIDs, including the human leukocyte antigen (*HLA)* locus [57], which is a region in the genome encoding six different HLA proteins [60]. Variations in the HLA locus have been implicated in several different diseases, including arthritis and other autoimmune disorders [60], due to the fact that the HLA locus protein products control antigen presentation [57], which is an essential role of the immune system.

## Arthritis

Arthritis is a public health concern because it is a major cause of disability in adults and is associated with high costs, thus increasing the financial burden on society [61]. Many different forms of arthritis exist, but in general, the overall disease is characterized as an “inflammatory joint disease”. Arthritis may be classified as acute, in that it arises from either a bacterial or viral infection, or it can be classified as chronic, which is often more debilitating. Individuals with chronic arthritis are typically worse off than those with the acute form because they may experience a reduction in their quality of life, various forms of disability, and an increased risk of early death. The financial burden of this disease is high [62], estimated at $128 billion annually [61], and has been predicted to increase as the population ages [62].

In the United States, arthritis is one of the most prevalent diagnosed health conditions. Among individuals with arthritis, 23.7 million adults are predicted to have limitations in terms of their activity and movements [63]. Hootman et al. performed a study in 2016 to update the prevalence of arthritis using baseline data from the 2010-2012 National Health Interview Survey. The prevalence of arthritis from 2010-2012 was 52.5 million adults in the US (22.7%). They projected by 2040, this will increase by 49%, up to 78.4 million adults (25.9%) [64]. The prevalence of arthritis varies by state (19-36%) and county (16-39%) [63]. Boring et al. assessed the prevalence of this disease in both rural and urban areas in the US and estimated that the prevalence of arthritis was 31.8% in rural areas and 20.5% in urban areas [63].

Several risk factors that may increase an individual’s risk of developing arthritis, are described in Table 5 [65].

Table 5. Factors associated with an increased risk of developing arthritis.

|  |  |
| --- | --- |
| **Risk factor** | **Explanation** |
| Age | As age increases, the risk for developing arthritis also increases. |
| Sex | More common in women (52%). |
| Genetics | Some genes are associated with a higher likelihood to develop specific types of arthritis such as rheumatoid arthritis. |
| Obesity | Specific to arthritis in the bones of the knee, extra weight can increase the onset and progression. |
| Joint injuries | Damage specific to a joint can cause arthritis in the bones of that particular area. |
| Infection | Some microbes can infect joints and therefore cause different types of arthritis. |
| Occupation | Jobs that involve excessive bending can increase the risk for developing osteoarthritis; extreme amounts of pressure on the joints can also lead to an increased risk of arthritis. |

As stated in the above table, some genes increase the risk for the development of different types of arthritis. Of importance, there is an interplay between genetics and epigenetics in two forms of arthritis: rheumatoid and psoriatic arthritis. Rheumatoid arthritis (RA) is an autoimmune disease that affects the joints, but also causes immune cells to attack host epitopes [66]. The heritability of RA is estimated to be ~60% [67]. MacGregor et al. conducted a study to investigate twin concordance for RA. They analyzed data from two published nationwide studies done in Finland and the UK in which heritability was analyzed by variance component analysis. They found the heritability of RA to be 65% in the Finland dataset and 53% in the UK dataset; thus they concluded that genetic factors likely contribute to RA [68]. Additionally, because of the development of genome-wide association studies (GWAS), several loci have been implicated as risk factors for RA. As of 2012, 35 loci have been identified as contributing to disease, including loci related to Class II HLA [69]. Nakano et al. performed a study to describe the epigenetics related to RA. They isolated genomic DNA from six RA and five osteoarthritis FLS lines and evaluated the methylation structure using HumanMethylation450 chip, which was confirmed with pyrosequencing. Researchers found hypomethylated loci identified in key genes associated with RA, as well as hypermethylation. As expected, those genes that were hypomethylated showed an increase in gene expression. They suggest that differentially methylated genes associated with RA could contribute to the overall pathogenesis of the disease [70].

There are similar conclusions to RA for psoriatic arthritis regarding the interplay of genetics and epigenetics. Psoriasis is a chronic inflammatory disease that can be primarily described as an excess of skin cells, resulting in cutaneous, scaly plaques. About one-third of individuals with psoriasis may develop psoriatic arthritis (PsA). Loft and colleagues performed a study using a candidate gene approach whether they looked at 53 SNPs in 37 genes that are related to the regulation of inflammation. They assessed 480 patients with psoriasis of which 151 had cutaneous psoriasis, 459 patients with psoriatic arthritis and 795 controls. They located “11 polymorphisms in 10 genes normally associated with psoriasis and/or cutaneous psoriasis and/or psoriatic arthritis, (p<0.05).” Once researchers corrected for multiple testing, two SNPs were identified as being significant, one of which was associated with psoriatic arthritis. This SNP, rs361525, is located in TNF genes [71]. In regards to epigenetics, Pollock et al. performed a systematic review of the existing literature to understand the current understanding of how this may contribute to disease pathogenesis. They looked at 52 articles, which described the most common epigenetic modifications in PsA, which included: DNA methylation, origin effect, genomic imprinting, epigenetic modifying enzymes and histone modifications [72].

For the purposes of this essay, participants with any form of arthritis were used for analyses. The types of arthritis that were included are: rheumatoid arthritis, polyarthritis, pyogenic arthritis, and arthritis mutilans.Although arthritis involves the inflammatory process, it is more specifically inflammation of the joints [62], not inflammation specifically associated with the brain. Therefore “arthritis: yes versus no” may not serve as a meaningful proxy for the inflammation going on in the brain of HD patients. No direct measures of neuroinflammation were available in the the dataset. My mentor at the Huntington’s Disease Care, Education and Research center, Dr. Karen E. Anderson, suggested focusing on comorbidities that involve an inflammatory process. Arthritis is more prevalent than MS, especially in older individuals, and this dataset comprises older participants. Also, the sample size for individuals with arthritis was sufficient (n=377). Therefore; arthritis was selected as a comorbidity.

## Multiple sclerosis (MS)

Multiple Sclerosis (MS) is a “chronic immune-mediated demyelinating disease of the central nervous system” [73] and may also be classified as a neurodegenerative disease [74]. It affects about 400,000 individuals in the US and 2.1 million individuals across the globe. MS can affect an individual’s overall quality of life [75]. The cost associated with treatment and care of individuals with MS is high. There is a positive relationship between increasing costs of MS and increases in the severity of the disease [76]. Direct costs of medical care associated with MS can be upwards of 10 billion dollars a year [75].

It is characterized by neurological episodes that come in a relapsing-remitting pattern because an individual’s immune system is attacking itself [77]. More specifically, in MS the immune system attacks the myelin that protects nerve fibers. This process ultimately leads to the deterioration or damage of nerves, affecting the relationship between the brain and body [78]. Subsequently, the white and grey matter in the brain is demyelinated leading to neurodegeneration [79]. Depending on the amount of degradation and location of damage, also known as lesions, patients show different signs and symptoms of the disease [78]. Some symptoms associated with MS include, but are not limited to, compromised mobility, weakness, depression, pain, intellectual impairments and problems with swallowing and/or breathing [75]. The overall neurological characteristic of the disease is neuronal death, which progressively gets worse over the course of the disease [80].

Immune cells, such as macrophages and circulating monocytes, function to maintain the balance of the immune system in the body and are involved in inflammatory responses in the brain (neuroinflammatory processes). These cells have been implicated in the brain pathology of some autoimmune diseases, including MS, and degenerative diseases [81]. Also, a recent study by Ponath and colleagues found that astrocytes also play a role in the formation and development of MS-related brain lesions [82].

The prevalence of MS, specifically those that are commercially insured, in the United States between 2008-2012 was determined by Dilokthornsakul, et al. who performed a retrospective study of a nationwide claims database, PharMetrics Plus, with over 42 million individuals represented. They determined the prevalence of MS in 2012 to be 149.2 per 100,000 individuals, which stayed the same from 2008-2012. They also state that from their analysis, women were 3.13 times more likely to have MS versus men [83]. In 2013, Kingwell and colleagues did a widespread literature search (n=123 studies) of European populations studies that were conducted between 1985 and 2011. Similar to Dilokthornsakul et al., Kingwell and colleagues state that prevalence was higher in women (3:1 ratio) compared to men [84]. Additionally, O’Gorman et al. state that there is a higher incidence of MS in female compared to males [80].

Both genetic and environmental factors and their interactions influence the pathology of MS [73]. Environmental factors that are associated with increasing one’s risk for MS include smoking, childhood obesity, low vitamin D levels, infection with the Epstein-Barr virus, potentially a high salt diet [82] and latitude [85, 86]. These are further described in Table 6.

Table 6. Factors associated with an increased risk of developing MS.

|  |  |
| --- | --- |
| **Risk Factor** | **Explanation** |
| Smoking | Increased susceptibility; risk ratio of 1.48 [87]  Smoking affects DNA methylation on a genome-wide level [88] |
| Childhood obesity | Exact mechanism poorly understood, has to do with microbiota of the gut and increased leptin levels- changes in these help to regulate the immune system [89].  Higher leptin levels may drive the immune system into a pro-inflammatory state [89]. |
| Vitamin D | Effects on both parts of the immune system- innate and adaptive [86]  Levels might effect risk, severity or persistence of Epstein-Barr virus [86]  Low levels of vitamin D increase risk- receptor for vitamin D acts as transcription factor. Once activated, acts in protein regulation including proteins involved with the immune system [90].  Higher levels- lower risk of developing MS; increasing exposure, smaller amount of relapses [91] |
| Latitude | Risk for MS decreases with higher latitude [85] |

The genetics of MS is complex in that multiple genes and signaling factors are involved. Sadovnick et al. state there is a strong genetic component to MS. They report that an increase in familial risk may be 300 fold for monozygotic (MZ) identical twins and 20-40 fold for first-degree relatives [92]. Willer et al. performed a “longitudinal population-based study of twins with multiple sclerosis (MS) in Canada” (n=370). Researchers report the concordance rates as: 25.% for monozygotic (MZ) twins, 5.4% for dizygotic twins (DZ) and 2.9% for their non-twin siblings. They also report that they observed a two-fold increase in the risk of MS when comparing dizygotic twins and non-twin siblings, but this difference was not significant [93]. Sadovnick and colleagues used a Canadian population-based sample with 16000 cases of MS to explore the genetics of MS with half siblings. They report that the rate of MS in half-siblings (n=1839) was 1.32 percent versus full-siblings (n=1395), which was 3.26 percent (p<0.001) [92]. Overall, there is a strong familial association seen in MS [94]. Sadovnick and colleagues report that the risk of disease for relatives increases with increasing relatedness, as supported by their analyses [92]. Another study performed by Sadovnick and colleagues calculated recurrence risk with 815 index cases and over 3000 of the index case’s siblings. They report the risk for relatives in developing MS is 3-5% [95].

Several immune signaling factors have been reported to play a role in MS, which include: TYK2, CD40, TNFAIP3, PTPN22, IL-2, and IL-2RA [58]. The most prominent loci involved in MS is the major histocompatibility complex (*MHC),* on chromosome 6p21.3 [96]. The development of new technology, such as genome-wide association studies (GWAS), has implicated several other loci in the susceptibility of MS; 110 MS risk variants have been identified in European populations [96]. In 2011, the International Multiple Sclerosis Genetics Consortium (IMSGC) worked with the Welcome Trust Case Control Consortium 2, on a GWAS, in which they recruited ~10,000 MS cases and 20,000 healthy controls, all of European descent, with the goal of finding significant loci that contribute to MS susceptibility. From this study, researchers were able to identify 52 significant loci as contributing to the genetics of MS. They report that most of these genes have some sort of immune function [97]. In 2013, the IMSGC performed another large multi-center study involving over 80,000 participants, also of European descent, in which they analyzed samples using an ImmunoChip. Researchers reported 48 new, significant, variants associated with a risk of MS [98].

For the purpose of this essay, all participants with MS were included, even though it was a small sample size, n=22. As described above, MS is a disease of the central nervous system that is mediated by the immune system [73]. This includes the degradation and/or damage of nerves [78], which is why it is also considered to be a neurodegenerative disease [74]; there is a demyelination of white and grey matter in the brain subsequently leading to neurodegeneration [79]. With this inflammatory disease, although the immune system and brain are involved, it is unclear whether this neurodegeneration can be associated with the same type of degeneration observed in the brains of HD patients. Therefore “MS: yes versus no”, although a better proxy than arthritis, still may not serve as an accurate representation of neuroinflammation and inflammation associated with HD. As stated previously, there were no direct measures of neuroinflammation in the dataset. Therefore, my mentor and I decided on using MS, prior to our inclusion of arthritis, because it involves an inflammatory process and subsequent neurodegeneration.

# Public health significance

I worked at the Huntington’s Disease Care, Education and Research Center in Washington, D.C. under the leadership of the director Dr. Karen E. Anderson. I was also able to work with the center’s social worker, Hope Heller, and research assistant, Natasha Scott. Additionally, I worked with Dr. Jan Blancato, the center’s genetic counselor and a cytogeneticist. I was tasked with creating my own research project, which I later transferred into my MPH essay. I was also able to observe various study and HD clinic visits. It was during one of these study visits that I met an HD patient, who also had MS. We discussed his life prior to the onset of MS, how this changed when he started developing symptoms of MS, and what his life looked like after he had developed symptoms of both MS and HD. I wanted to further my understanding of the implications of two chronic illnesses, how to manage comorbidities along with HD and if there was a possibility to extend the functional lifespan of people with HD. This patient became the inspiration for my masters’ essay; I wanted to explore the potential relationship between HD and inflammatory diseases such as MS to gain insight into a potential relationship and to best to manage comorbidities along with a HD diagnosis.

The review of the literature revealed several important public health issues related to HD and comorbidities. The most studied populations involve those of European descent, that is, individuals from the United States and European countries. Fewer studies have been done in other populations. The prevalence of HD is reported to be highest among populations of European descent, but there may be an underlying bias. More research has been done in these populations, so people would be more likely to be diagnosed with HD, resulting in a higher estimate of prevalence in these populations compared to other populations. Studies involving Asian, and African American populations report a lower prevalence, which also could be biased by the fact that these populations are not studied as frequently. More studies and research on HD needs to be done in these understudied populations to obtain better estimates of prevalence of HD.

Another issue of public health importance is the lack of adequate long-term care facilities available for HD patients. Costs of treating and caring for HD patients is high because family members and caretakers are turning to in-home care, which can be expensive. There is a lack of support and care for these patients. There is a shortage of facilities or specific units in these facilities specifically dedicated to HD patients. Also, many long-term facilities refuse to take HD patients because, depending on their symptoms, some HD patients can be difficult; they can become irritable and aggressive, even towards other residents in the facility.

Finally, knowledge of relationships between HD and other comorbidities is lacking. The potential for an interaction between HD and other disorders is possible, but a relationship, if any, is poorly understood. Results from this research would facilitate an improved understanding of the underlying mechanisms that, in turn, may lead to methods of treatment and improved care for these complicated patients.

# Specific aims

Several studies, and potential treatment modalities, indicate a possible relationship between progression of Huntington’s disease and inflammation, especially neuroinflammation. The overall goal of this study was to assess the possible relationship between Huntington’s disease (HD) and neuroinflammation using data from a large, observational, multicenter, multi-national cohort study called Enroll-HD. Because direct measures of neuroinflammation were not available in the dataset, I used the presence of two chronic inflammatory disorders, Multiple Sclerosis (MS) and arthritis, as proxies for the presence of inflammation. Specifically, I first assessed the demographic and risk factor differences between participants with manifest HD versus pre-manifest HD versus genotype negative individuals versus family controls. Then I assessed differences in chronic inflammatory disorder prevalence between these groups, as well. Because other factors (such as smoking and alcohol) may influence progression of HD, I also assessed these variables.

**Specific Aim 1:** Characterize the demographics of Enroll-HD cohort overall and by the four HD subgroups: individuals with manifest HD disease, individuals with pre-manifest HD, individuals who are genotype negative and family controls.

Assess whether demographics differ among HD subgroups.

**Specific Aim 2:** In Caucasians, participants of Enroll-HD, characterize the differences in risk factor distributions including sex, age, number of CAG repeats, CAP score, smoking and alcohol use among the four HD subgroups (individuals with manifest HD, individuals with pre-manifest HD, individuals who are genotype negative and family controls).

Determine if differences in risk factors for HD (age, CAG repeats, CAP score) by HD subgroup are consistent with previous reports.

Assess whether prevalence of smoking or alcohol use varies by HD subgroups.

**Specific Aim 3:** Assess possible differences in chronic inflammatory disorder prevalence among the four HD subgroups (individuals with manifest HD, individuals with pre-manifest HD, genotype negative and family controls).

a. Test for differences in prevalence of MS and arthritis, by HD subgroup.

# Study design

## Dataset

The dataset used in this study is a prospective, observational, multicenter, multi-national cohort study called Enroll-HD [99]. This dataset is global, in that all center locations where data is collected locally are compiled into one large database. Georgetown University’s IRB in Washington, D.C and the University of Pittsburgh provided the approval for the use of this dataset (see Appendix B). The Enroll-HD study does not limit the number of subjects, it aims to recruit all subjects who meet the eligibility criteria and agree to join, with the overall goal of enrolling one-third of the affected HD population in each study region (North America, Latin America, Europe, Asia, and Australia/New Zealand) [99]. Both males and females 18 years and older are eligible and there are no restrictions on ethnicity or race. An exclusion criterion is that those individuals with a choreic movement disorder, but test negative for the HD expansion are excluded. Recruitment of individuals for Enroll-HD involves various methods. Patients with HD and family members are recruited from specialty clinics including Human Genetics, Neurology and Psychiatry. Additionally, neurologists and community clinics that may see HD patients recruit individuals for the study. Once participants are declared as eligible, they are consented for the study and their consent is updated at each annual visit, because this is an ongoing study. Individuals who join Enroll-HD are asked to talk to and forward information to their relatives. Lastly, individuals may learn more about the study and become interested via the Enroll-HD website, clinical practices, support groups, and advocacy newsletters [99].

For this study, only data from the baseline visit was used. The study sample came from various study centers located in North America, Latin America, Europe, Asia, Australia and New Zealand [99]. Individuals were classified as having HD if they carried the CAG repeat expansion (>40) in the HD gene [99]. These individuals may be in two different stages of the disease, pre-manifest HD or manifest HD. Those who are pre-manifest were not showing symptoms at the time of enrollment and those who are manifest HD were showing symptoms at the time of enrollment.

Two types of controls for Enroll-HD are available. “Genotype negative” controls are individuals who come from a family with HD (family members), who were at risk for HD, but after testing they have a number of CAG repeats in the normal range (<27 or 27-35 repeats). “Family controls” are individuals who did not grow up in a family affected by HD; they are not members of a HD lineage. For example, they may be a father of an HD patient, but the patient inherited the HTT gene from the mother [99]. Additionally, family controls may not be genetically related to the HD participant; for example, they may be a spouse of an individual affected with HD. Subjects who were recruited for the study were put into six categories when they enrolled, which included manifest HD, pre-manifest HD, genotype unknown, genotype negative, family control and community control [99]. Community controls are defined as individuals who are recruited from the population and have no relationship to an affected individual, either familial or through marriage. These participants are rare and therefore, not included in this dataset. In the compilation of the Enroll-HD data from all locations, the global dataset puts all participants into four clear categories, which are described in Table 7 [99]. These are the categories used to define the population for this study.

Table 7. Category and accompanying description for participants.

|  |  |
| --- | --- |
| **Category:** | **Description:** |
| Manifest HD | Carriers with clinical features of HD |
| Pre-manifest HD | Carriers without clinical features of HD |
| Genotype negative | First or second degree relative who has undergone testing and test negative for the HD expansion |
| Family controls | Unrelated by blood to HD carriers, and did not grow up in a family affected by HD (i.e. spouse) |

## data collection

This study is observational; there is no experimental treatment or intervention given. The study period for Enroll-HD is open-ended and participants are asked to come for annual study visits; they are asked to come to as many of these as possible. At each study visit, several assessments are done which include mandatory and optional assessments. Mandatory core assessments include motor, cognitive and behavioral testing. There are also extended and optional assessments, which include surveys [99].

Motor assessments are done with HD patients because this is a clear, phenotypic way for physicians to measure the progression of HD. To evaluate an individual’s motor function, a standardized, 15-part motor exam using the Unified Huntington’s Disease Rating Scale (UHDRS) is done [12, 100]. The UHDRS results in an outcome known as the total motor score (TMS), an overall assessment of the movement of the patient, and is most likely due to chorea [100]. It comprises five domains surrounding motor impairment; each individual item is “rated on a 5-point scale ranging from 0 (normal) to 4 (severest impairment)” [1]. The five domains that are measured during the motor exam include: “eye movement, chorea (jerky movement), dystonia (muscle spasm and twisting), bradykinesia (slowness in movement) and rigidity (stiffness)” [1]. The TMS is calculated as a summation of each of the 31 items that are measured [1]. After this exam, a “certified motor rater”, usually the physician on-hand who administers the motor exam, assigns a score according to the diagnostic confidence level (DCL). This is used to ask whether the individual in question “meets the operational definition of the unequivocal presence of an otherwise unexplained extrapyramidal movement disorder in a subject at risk for HD”. This scale ranges from 0-4 and the numeric designations correlate to a definition of impairment, which range from, 0 being unimpaired, 1 being non-specific motor impairments (less than 50% confidence) to 4 being motor impairments that are clear signs of HD (99% confidence) [13]. In the Enroll-HD study visits, the clinician who administers the motor exam also assigns the DCL to that patient [100].

Another test that is done during study visits is a functional assessment using the UHDRS functional assessment scale and UHDRS independence scale [99, 100]. This involves various questions administered by the clinician, usually the same individual who did the motor assessment. These questions address topics such as occupation, finances, domestic chores, activities of daily living, and one’s care level, which results in a UHDRS total functional capacity (TFC) score [100]. The TFC is considered a standard assessment in terms of overall function of a patient with HD. It has been shown to be a reliable measurement in documenting the progression of HD. This score ranges from 0-13, 0 being a complete loss of function to 13 being normal function [14].

The behavioral assessment is the Problem Behaviors Assessment-Short (PBA-s) and measured using several scales including Hospital Anxiety/Depression Rating Scale (HADS); Snaith Irritability Scale (SIS); Columbia Suicide Severity Rating Scale (CSSR) [99]. The PBA-s measures the frequency and severity of symptoms [101]. Questions in this assessment include those directed at assessing: “depressed mood, low self esteem, anxiety, suicidal thought, aggressive behavior, irritability, preservation, compulsive behaviors, delusions, hallucinations and apathy” [99]. The answers to these questions are rated by the interviewer based on the frequency and severity of this particular behavior the participant may or may not have had in the last month. If necessary, several extended behavioral assessments may be performed which include Hospital Anxiety/Depression Rating Scale (HADS); Snaith Irritability Scale (SIS); Columbia Suicide Severity Rating Scale (CSSR) [99]. The HADS is self-reporting and is used to rate symptoms associated with depression and anxiety that pertain to that individual’s mood. The scale consists of 14 items evenly split between anxiety and depression in which each item is rate with a four-point scale; numbers are totaled for each section (anxiety and depression) [99]. This scale is useful in gauging the severity of both anxiety and depression in an individual [102]. The SIS is a self-reporting assessment of irritability [103] that has eight items, four gauging inner irritability and four gauging outer irritability; each is rated on a four-point scale [99]. Finally, the CSSRS was developed by the National Institute of Mental Health Treatment of Adolescent Suicide Attempters Study in order to better measure the severity of suicidal events as well as monitor these incidents [104]. This scale aims to get a better idea of suicidal ideations and behavior to rate the degree of suicidality [99].

The core cognitive assessments are done to assess the patient’s cognitive ability at the time of the visit. These include the symbol digit modality test (SDMT), Stroop color naming test, Stroop word reading test, and categorical verbal fluency test [105]. If time permits, there are several extended cognitive assessments that can be given. These include the Stroop interference test, the trail making tests, and the mini-mental state examination [99]. These various assessments aim to test an individual’s verbal fluency, substitution skills, and overall neuropsychological ability at the time of each visit. The SDMT evaluates the substitution skills of a participant. They are given a key at the top of the page, in which they have 90 seconds to match each figure with a number. The total score depends on how many answers are correct in these 90 seconds. Both the Stroop color naming test and Stroop word reading test are commonly used to evaluate cognition. These tests require participants to name colors, such as red, green, and blue, and then read what the word says in regards to color, not the actual color the word is given in. For example, the word may say blue, but it would be written in green ink; participants would have to say blue to get this question correct. The final assessment is the categorical verbal fluency test that assesses an individual’s ability to spontaneously produce words orally pertaining to a particular category. For example, determining how many words a participant can say in regards to animals that start with the letter F. This is done within 60 seconds and each correct answer constitutes a point; the total points are equivalent to that person’s cognitive performance [99, 106].

At each participant’s baseline visit, blood samples are collected to assess the number of CAG repeats present in that individual. Up to 40mL of peripheral blood is collected at each visit that is stored in a central biospecimen repository (BioRep). 10mL of that blood is collected in a tube containing acid citrate dextrose solution and shipped by DHL to the central biorepository facility. The sample is labeled with a unique Huntington’s disease identifier number (HDID) and DNA is extracted using standard procedures. Routine quality control checks were done to determine the quality and integrity of the DNA in the sample. The DNA is genotyped according to standard procedures, which includes measuring the CAG repeat size using two sets of primer pairs [1]. All of the study data is entered into each local Enroll-HD database, then summarized across all sites to create a global Enroll-HD dataset. Other descriptive data was collected at each baseline visit included self-report of socio-demographic information, medical history, comorbidities, current therapies, smoking habits, alcohol use, ethnicity, and sex [99].

The variables used in this study are a compilation of several variables that are present in the global Enroll-HD dataset (Table 8). Variables specific to HD that were examined, but not used in this data analysis, are listed in Appendix A, Table 14. The CAP score, as previously mentioned, is the probability of an individual being diagnosed with HD within a specific time frame [21]. First, 33.66 is subtracted from the number of CAG repeats present in an individual to act as a correction factor accounting for error in the CAG repeat measure [21]. Then the product of this value is multiplied by the age of the participant and then divided by a constant (432.33). This results in a CAP score that ranges from <1 to >1 and is interpreted as the probability of an individual being diagnosed with HD within 5 years [21].

Table . Description of variables used from the Enroll-HD dataset for this essay.

|  |  |
| --- | --- |
| **Variable:** | **Description:** |
| region | Region where the participant’s local site is; where they went for their annual study visits |
| sex | Biological sex of the participant |
| race | Race of the participant |
| age | Age of participant at baseline visit |
| hdcat | HD category  2= pre-manifest, pre-motor-manifest HD  3= manifest motor-manifest HD  4= genotype negative  5= family control  Note: categorical variable |
| cag | Number of CAG repeats, the higher repeat out of the two alleles present  Note: continuous variable |
| caps | CAP score: probability of being diagnosed with HD [21]  CAPs= (age x (CAG-33.66)/432.33)  < 0 = not likely or far away from diagnosis, < 50% chance  =1 50/50 chance of developing in 5 years  >1 = >50% chance of developing in less than 5 years  Note: continuous variable |
| inflamm | Inflammatory comorbidities  0= none  1= arthritis  2= Multiple Sclerosis (MS)  Note: categorical variable |
| iinflamm | Indicator variable for inflammation  0= not having an inflammatory disease  1= having inflammatory disease- only including arthritis and MS  Note: binary variable |
| alcoh | Does the participant currently drink alcohol?  0= no  1= yes  Note: binary variable |
| smoke | Does the participant currently smoke?  0= no  1=yes  Note: binary variable |
| alcohu | Units of alcohol per week  Note: continuous variable |
| smokeu | Units of packs per year  Note: continuous variable |

# Statistical Methods

Basic descriptive statistics (means, standard deviations, and/or frequencies) of the study population were calculated for the four Enroll-HD subgroups: manifest HD, pre-manifest HD, genotype negative and family controls. Frequency histograms of age and number of CAG repeats were plotted for each subgroup using StataSE version 14. In addition, the relationship between number of CAG repeats and CAP score was plotted for all participants using the software JMP Pro13.

Because greater than 87% of the study population self-identified as Caucasian ancestry, all subsequent analyses were performed on this subset. Analyses of variance was done to assess differences among HD subgroups for quantitative measures including age, number of CAG repeats, CAP score, number of alcohol units (number of drinks per week), and smoking units (number of packs smoked per year). For categorical data (alcohol use, smoking use, and inflammatory disease comorbidities), differences among the four HD subgroups were assessed using chi-square analyses. If a significant p-value for group differences was observed, follow-up analyses comparing subgroups were done also using chi-square analyses. All analyses were unadjusted and did not control for relatedness between individuals. All statistical analyses were performed in StataSE version 14.

# results

## Basic demographic characteristics and measurements of hd risks overall participants and by participant category

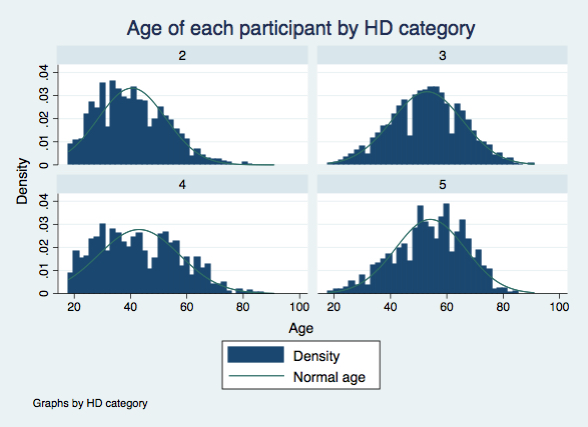
As shown in Table 9, over half of the participants in Enroll-HD lived in Europe (55% overall) or Northern America (41% overall) and 93% of participants self-identified as being of Caucasian ancestry. Population characteristics specific to HD are shown in Appendix A, Table 15.

Table . Population characteristics of Enroll-HD dataset.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Characteristic | Category of participant | | | | |
| Manifest HD  (n=4,752) | Pre-manifest HD (n=1,862) | Genotype negative (n=1,089) | Family control  (n=1,011) | Total  (n= 8,714) |
| Mean(SD) % | Mean (SD) % | Mean(SD) % | Mean(SD) % | Sum (all groups) |
| Region |  |  |  |  |  |
| Australia | 3.03 | 6.44 | 3.31 | 2.37 | 4% |
| Europe | 64.48 | 52.69 | 37.10 | 35.91 | 55% |
| Latin  America | 0.69 | 0.16 | 0.92 | 0.49 | 0.6% |
| Northern  America | 31.80 | 40.71 | 58.68 | 61.23 | 40% |
| Female sex | 51 | 61 | 66 | 56 | 55% |
| Race |  |  |  |  |  |
| Caucasian | 94.15 | 93.76 | 87.42 | 91 | 93% |
| American  Black | 1.09 | 0.54 | 1.65 | 0.89 | 1% |
| Hispanic | 1.58 | 1.24 | 2.85 | 2.87 | 2% |
| American  Indian | 0.42 | 0.81 | 3.40 | 1.68 | 1% |
| Asian | 0.65 | 0.59 | 0.55 | 1.38 | 0.7% |
| Other | 2.11 | 3.07 | 4.13 | 2.18 | 3% |
| Age, years | 52.98 (12.59) | 40.52 (12.01) | 43.04 (14.38) | 54.14 (12.41) | 49.2 (13.9) |
| # of CAG repeats | 44.02 (3.80) | 42.42 (2.78) | 20.28 (3.67) | 20.07 (3.42) | 38 (10.6) |
| CAP score | 1.19 (0.23) | 0.7783 (0.213) | -1.33 (0.58) | -1.70 (0.59) | 0.45 (1.17) |
| Inflammation (yes/no) | 4.0 | 4.19 | 4.96 | 7.62 | 5% |
| Inflammation |  |  |  |  |  |
| Arthritis | 4 | 4 | 4.5 | 7.55 | 4.33% |
| MS | 0.2 | 0.4 | 0.5 | 0.1 | 0.25% |
| Total | 4.2 | 4.4 | 5.0 | 7.65 | 4.58% |
| Alcohol use (yes/no) | 38 | 60 | 53 | 53 | 46% |
| Alcohol use, week | 45.71 (30.71) | 45.93 (30.31) | 42.49 (30.26) | 45.96 (31.29) | 45.4 (30.6) |
| Smoking (yes/no) | 27 | 25 | 21 | 18 | 25% |
| Pack of smokes per year | 136.9 (79.58) | 128.4 (89.66) | 128.8 (92.98) | 130.4 (84.70) | 133.6 (83.8) |

**Table 9 Continued**

The distribution of age within each participant category ranged from 18-91 years and varied between the HD subgroups (Figure 2). The distributions were as expected; the pre-manifest HD group (group 2) had a higher frequency of younger participants, whereas the manifest HD group (group 3) had a higher frequency of middle-aged participants.



**Key:**

2= pre-manifest HD

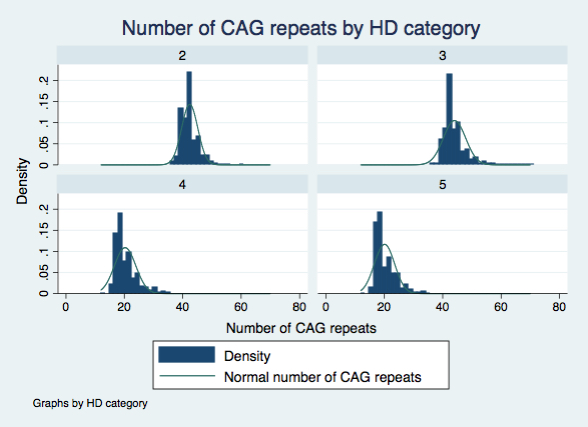
3= manifest HD

4= genotype negative

5= family controls

Figure . Histograms of age of participants for each HD category.

The distribution of the number of CAG repeats for each HD subgroup were plotted (Figure 3). As expected, the number of CAG repeats were similar for family controls (range 13-35 repeats; group 5) and individuals who tested negative for HD (range 12-35 repeats; group 4). The number of CAG repeats was also similar for individuals with pre-manifest HD or manifest HD (range 36-61 repeats, group 2; and 36-70 repeats, group 3, respectively).



**Key:**

2= pre-manifest HD

3= manifest HD

4= genotype negative

5= family controls

Figure . Histograms of number of CAG repeats for each category; groups 2 and 3 show a higher number of repeats when compared to groups 3 and 4.

Figure 4 plots the relationship between the number of CAG repeats and CAP score, using a box and whiskers plot. For each CAG repeat category, the median, interquartile range, and 95% upper and lower limits are plotted. The dots on the figure are outliers of the 95% confidence interval. As expected, individuals who have fewer than 35 repeats have a CAP score less than or equal to zero, indicating they will likely not develop HD. The negative CAP score indicates that these individuals are very far away from a diagnosis, i.e. likely never to have HD. Individuals with a CAP score equal to 1 have a 50/50 chance of developing HD in five years; whereas those with CAP scores greater than 1 have a greater than 50% chance of developing HD in less than five years. As the CAP score increases, the time to diagnosis decreases [21]. Additionally, as the number of CAG repeats increase the CAP score also increases. This plot illustrates that the relationship between the number of CAG repeats and probability of developing HD is not linear. Also, for a specific number of CAG repeats, the CAP score (which includes an age component) varies widely, thus indicating that the probability of HD diagnosis within the next 5 years, also varies widely.

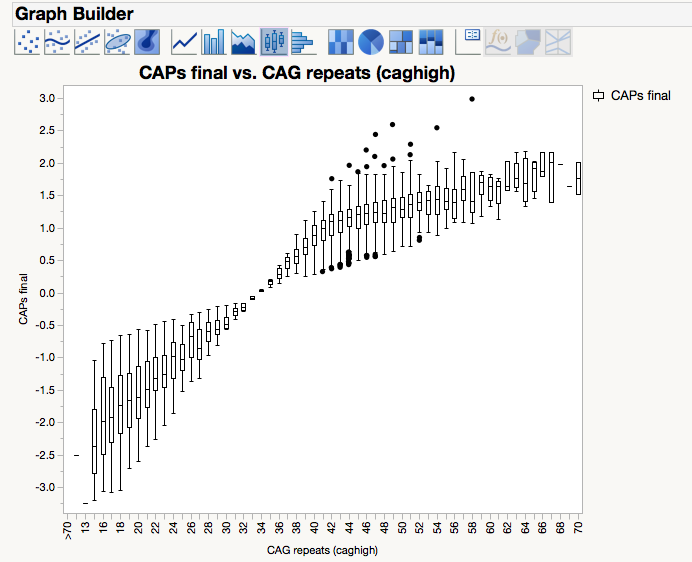


Figure . Relationship between number of CAG repeats and CAP score, based on 8,714 participants.

## Assessment of demographic variables and hd risk factors among four participant categories in caucasians only

Because the sample sizes for individuals in non-Caucasian ancestry groups were small (ranging from 0.55 to 3.5% of the population, see Table 9), all subsequent analyses were performed using data on individuals who self-identified as having Caucasian ancestry (Table 10).

Table . Demographics among four participant groups in Caucasians.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Category of participant | | | | |
| Characteristic | Manifest HD (n=4,473) | Pre-manifest HD (n=1,744) | Genotype negative (n=952) | Family control (n=920) | Total  (n=8,089) |
|  | Mean(SD) % | Mean (SD) % | Mean(SD) % | Mean(SD) % | Sum (all groups) |
| Female sex | 50 | 61 | 67 | 55 | 55% |
| Age, years | 53.17 (12.59) | 40.69 (11.98) | 43.31 (14.39) | 54.50 (12.41) | 45.77 (14.0) |
| # of CAG repeats | 43.96 (3.80) | 42.38 (2.78) | 20.29 (3.67) | 20.10 (3.42) | 38.11 (10.46) |
| CAP score | 1.19 (0.23) | 0.7790(0.213) | -1.34 (0.59) | -1.71 (0.60) | 0.47 (1.16) |
| Alcohol use (yes/no) | 38 | 60 | 53 | 54 | 46% |
| Alcoholic drinks/week | 45.68 (30.69) | 45.91 (30.20) | 42.80 (30.18) | 45.98 (31.42) | 44.66 (31.17) |
| Smoking (yes/no) | 27 | 26 | 20 | 18 | 25% |
| Cigarette packs/year | 137.3 (79.48) | 128.1 (89.25) | 127.0 (93.18) | 129.0 (83.25) | 134.49 (89.35) |
| Inflammatory Disease |  |  |  |  |  |
| Arthritis | 3.78 | 3.90 | 4.2 | 7.39 | 4.27% |
| MS | 0.2 | 0.4 | 0.42 | 0.11 | 0.3% |

ANOVA of continuous risk factors revealed significant differences among participant groups by age (p< 0.0001), CAG repeats (p< 0.0001) and CAP score (p< 0.0001), see Table 11. Participant groups did not differ by number of alcoholic beverages consumed per week (p=0.2360) and packs of cigarettes smoked per year (p=0.1133). As expected, individuals who were pre-manifest HD were younger than those with manifest HD. The number of CAG repeats also differed significantly among groups (p<0.0001). Those with either pre-manifest HD or manifest HD had a higher number of repeats than those who were genotype negative for HD and family controls, 42-44 repeats versus 20 repeats, respectively. The number of CAG repeats did not differ between genotype negative and family controls, as expected (p=0.2659). Significant differences between groups were observed for the CAP score (p<0.0001). Pre-manifest HD and manifest HD were expected to have a higher probability of developing HD than genotype negative and family controls, which is what was observed. There was no significant difference between groups for number of drinks consumed per week (p=0.2360), and packs of cigarettes smoked per year (p=0.1133). Subsequent comparisons of pre-manifest HD versus manifest HD participants for packs of cigarettes smoked per year revealed a significant difference between subgroups (p=0.0444).

Table 11. Summary of p-values from tests of continuous variable risk factor (ANOVA) differences between HD subgroups in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
| **Analysis of Variance P-values for HD Subgroup Comparisons** | | | |
| **Variable** | **Test of HD Case Subgroups** | **Test of HD Control Subgroups** | **Test of all 4 Subgroups** |
| Age | P<0.0001 | P<0.0001 | P<0.0001 |
| CAG repeats | P<0.0001 | P=0.2659 | P<0.0001 |
| CAP score | P<0.0001 | P<0.0001 | P<0.0001 |
| Alcohol units | P=0.8439 | P=0.1033 | P=0.2360 |
| Smoking units | P=0.0444 | P=0.8306 | P=0.1133 |

Chi-square analyses of categorical risk factors are shown in Table 12. Overall, the percent of women participants differed significantly among all HD subgroups (p<0.001). In addition, the proportion of women participants between pre-manifest and manifest HD, as well as between genotype negative and family controls differed significantly. Alcohol consumption (measured as “yes/no”) differed significantly among all four HD subgroups (p< 0.001). Individuals who had manifest HD had the lowest levels of alcohol use, whereas individuals with pre-manifest HD had the highest levels (38 versus 60% respectively). Sub-analyses of individuals with pre-manifest versus manifest HD revealed that this difference was significant (p< 0.001). However, there was no statistically significant difference between genotype negative and family controls (p= 0.960). Smoking frequency also differed significantly among participant categories (p<0.001); the highest percentage of smokers were in the manifest HD subgroup and the lowest percentage of smokers were in the family controls group (27% versus 18%, respectively). However, smoking did not differ between individuals with pre-manifest versus manifest HD (26% versus 27%), respectively; p=0.176), or between individuals who were genotype negative and family controls, (20% versus 18%, respectively; p=0.752). The full results of each chi-square analysis are shown in Appendix A, Tables 16-24.

Table 12. Summary of p-values from tests of chi-square analysis for categorical variable risk factors, between HD subgroups in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
| **Chi-Square P-values for HD Subgroup Comparisons** | | | |
| **Variable** | **Test of HD Case Subgroups** | **Test of HD Control Subgroups** | **Test of all 4 Subgroups** |
| Sex | P<0.001 | P<0.001 | P<0.001 |
| Alcohol consumption | P<0.001 | P=0.960 | P<0.001 |
| Smoking use | P=0.176 | P=0.752 | P<0.001 |

## Relationship of inflammatory disorders and hd category

Lastly, I assessed the possible relationship between HD category and (1) any inflammatory disease (yes/no) and (2) specific inflammatory diseases (arthritis and MS; Table 13). Overall, inflammatory disease (yes/no) was significantly different among all four HD categories (p< 0.001). Individuals who had manifest HD had the lowest prevalence of comorbidity with arthritis or MS and family controls had the highest prevalence (4% versus 7.4%, respectively). Further analyses revealed a significant difference between genotype negative and family controls (4.2% versus 7.4%, respectively; p=0.009). However, there was no significant difference between individuals with pre-manifest HD and individuals with manifest HD (p= 0.565).

The four groups were significantly different when stratified by arthritis, (p< 0.001), but not when stratified for MS (p=0.304). Family controls had the highest prevalence of arthritis and manifest HD had the lowest prevalence of arthritis (7.4% versus 3.8%, respectively). Although not significant, genotype negative individuals had the highest prevalence of MS and manifest-HD had the lowest prevalence of MS (0.42% versus 0.2%, respectively). Sub-analyses revealed no significant difference in arthritis prevalence between the HD case subgroups (p=0.812), but prevalence differed significantly between HD control subgroups (p=0.003). With respect to prevalence of MS, no differences were observed between HD case subgroups and HD control subgroups (p=0.161 and p=0.205, respectively). The full results for chi-square analysis for inflammatory diseases are shown in Appendix A, Tables 25-33.

Table 13. Summary of p-values from tests of chi-square analysis for categorical variable inflammatory diseases, between HD subgroups in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
| **Chi-Square P-values for HD Subgroup Comparisons** | | | |
| **Variable** | **Test of HD Case Subgroups** | **Test of HD Control Subgroups** | **Test of all 4 Subgroups** |
| Either CID (yes/no) | P=0.565 | P=0.009 | P<0.001 |
| Arthritis (yes/no) | P=0.812 | P=0.003 | P<0.001 |
| MS (yes/no) | P=0.161 | P=0.205 | P=0.304 |

# Discussion & Conclusions

In this study, I used the Enroll-HD cohort to first assess differences in demographics and risk factors in the dataset, and to then test the possible association between Huntington’s disease and neuroinflammation. I used the presence of two chronic inflammatory disorders, arthritis and Multiple Sclerosis, as proxies for the presence of heightened inflammation. I first created a population characteristics table and histograms of age and number of CAG repeats by HD subgroup to visualize the data. As expected, the mean age for pre-manifest HD was lower than the mean age for manifest HD. Individuals who test positive for the mutant *HTT* (>40 repeats) but do not show symptoms are typically younger than those who display symptoms (manifest HD). The mean number of CAG repeats also was similar in the pre-manifest HD and manifest HD groups (42 and 44, respectively), as expected. CAG repeats greater than or equal to 40 results in the development of HD [35]. Characteristics of genotype negative and family controls also were consistent with expectation; the mean number of CAG repeats was 20 in both groups. The average number of CAG repeats in the world population is 17-20 [22].

A box and whiskers plot was included to visualize the relationship between number of repeats and the probability of developing HD (CAP score) in this cohort. A higher CAG repeat length results in a higher probability of being diagnosed with HD and the CAP score is, partially, a function of CAG repeat length. I noted a narrowing in the graph around 32-38 CAG repeats, which is consistent with repeats ranging from 27-35 being considered of intermediate risk. Although this range of CAG repeats is non-pathological, the risk of transmitting an expanded CAG repeat sequence that falls within the range constituting HD is higher [107].

A second characteristic table was created in the Caucasian sample only (n=8,089) in an attempt to reduce possible heterogeneity; individuals who self-identified as being of Caucasian ancestry constituted greater than 87% of the population. Analysis of variance was used to assess differences among HD categories for continuous variables, including age, number of CAG repeats, CAP score, alcohol consumption (drinks/week) and smoking use (number of packs smoked/year). Significant differences among HD groups were observed for age, number of CAG repeats, and CAP score, but no differences for mean number of drinks consumed per week or packs of cigarettes smoked per year, (p=0.2360 and p=0.1133, respectively, Table 11). For example, the mean CAP score for each category differed significantly by decreasing severity of HD subgroup (1.19, 0.7799, -1.34 and -1.71, respectively). Thus, as expected, individuals with pre-manifest HD will eventually start to show signs and symptoms, but their probability of diagnosis within five years is not as high as those with manifest-HD at the time of enrollment (CAP score over 1.0). Also individuals who are genotype negative and family controls have negative CAP scores, because their probability of being diagnosed with HD is unlikely to occur.

Analyses of sex, drinking and smoking categories were done using chi-square tests to assess differences between HD subgroups (Table 12). The HD subgroups differed with respect to proportion of women, alcohol consumption and smoking use (p<0.001, for all three variables). The lowest levels of alcohol consumption (38%) and the highest prevalence of smoking (27%) were observed for individuals with manifest HD. Additional sub-analyses of sex showed a significant difference between HD case subgroups as well as HD control subgroups (p<0.001, for both analyses). The interpretation of this result is unclear, but may reflect a difference willingness to be tested between men and women. If at-risk women, who do not yet display symptoms, are more willing to be tested, then the frequency of women would be higher among pre-manifest HD individuals, as well as genotype negative individuals.

Sub-analyses for the prevalence of alcohol indicated a significant difference between groups for pre-manifest HD and manifest HD (p<0.001) but not for genotype negative and family controls (p=0.960). This outcome is consistent with the current literature, researchers have reported that the level of alcohol consumption is lower among individuals manifesting symptoms of HD. Patients with HD who consume alcohol are more likely to have the presentation of motor symptoms and behavioral problems as opposed to patients who do not [108]. Byars et al. described that a lifetime use of alcohol, especially alcohol abuse, was significantly associated with an earlier onset of HD [109]. Additionally, Ehret et al. reported that individuals who used, and potentially abused, alcohol after the onset of HD symptoms had psychiatric symptoms that progressively worsened [110]. In 2017, Schultz et al. performed a retrospective, observational study with the Enroll-HD dataset to assess substance abuse as it relates to the onset of motor symptoms in HD. Participants who abused alcohol (n=374) were compared to controls (n=692), a group of participants who had never abused substances. Researchers reported that onset of motor symptoms occurred ~1 year earlier among individuals with HD who abused alcohol versus controls (p=0.04) [111].

Smoking prevalence differed among HD subgroups (p<0.001). However, sub-analyses for smoking use did not indicate a significant difference between groups for pre-manifest HD and manifest HD (p=0.176), or between genotype negative and family controls (p=0.752), suggesting the difference between groups was due to differences between case and control status. The prevalence of smoking is typically higher among individuals who manifest symptoms of HD. Tariq et al. described nicotinic acetylcholine receptors as being potential targets for intervention, specifically for neurodegenerative diseases. Researchers observed the conservation of “striatal dopaminergic neurons” in rats models of HD, which had a protective effect in these7 experimental HD models [112]. Thus, low levels of alcohol consumption and high levels of smoking use in those with manifest HD may help in controlling symptoms of HD. The consistency of similar results for smoking and drinking in this cohort, in conjunction with previous reports, helps validate that the dataset used in this essay is representative of the overall HD population.

Lastly, the proxies used in this study for neuroinflammation (chronic inflammatory diseases including, arthritis and MS) were tested for differences in prevalence between HD subgroups. I hypothesized that individuals with manifest HD would have a higher prevalence of inflammatory diseases compared to pre-manifest HD, genotype negative and family controls. This hypotheses supported by evidence from the literature that neuroinflammation is part of the pathogenesis of HD [22]. I observed a significant difference between HD subgroups for both models of inflammation (p<0.001 for all); any chronic inflammatory disease (yes/no) and an independent test of arthritis, but not for MS (p=0.304), which is likely due to the small sample size (n=22). Demographics of the population (Table 10) revealed the level of MS to be higher in those with pre-manifest HD verses manifest HD (0.4% versus 0.2% respectively). However, the highest level of overall chronic inflammatory disorders was observed in family controls (7.5%). These results were contrary to my prediction.

Subsequent chi-square analyses of between group comparisons revealed no significant difference in inflammation (yes/no) for pre-manifest HD versus manifest HD (p=0.57), but there was a significant difference between genotype negative and family controls (p=0.009). Additionally, there was no significant difference for prevalence of inflammatory diseases (arthritis or MS) for pre-manifest HD and manifest HD, but the prevalence of disease differed between HD control subgroups for arthritis (p=0.003), but not for MS (p=0.205). Therefore, the significant difference across all subgroups for any chronic inflammatory disease (yes/no) and the independent test of arthritis is likely due by the high rate of inflammatory diseases in older individuals, especially arthritis. Upon further investigation, the family controls had the highest mean age (55 years), so it is likely that age is a contributing factor to the high levels of inflammatory disorders observed in this group; age was not controlled for when analyzing differences between chronic inflammatory disease groups.

The use of the Enroll-HD cohort was a strength of this study because it had a large sample size (n=8,714) overall [8,089 when limited to self-identified as Caucasians] and was representative of the general HD population. Additionally, this analysis only includes baseline data from 2013-2016, but longitudinal data comprising follow-up visits and check in phone calls is available.

The dataset and my analyses also had several limitations. First the dataset lacked a quantitative (continuous) measure of neuroinflammation, such as serum levels of C-reactive protein, interleukin-6 or inflammatory biomarkers in the CSF of patients. Assaying inflammatory biomarkers from the blood samples is not part of the Enroll-HD procedure. If these markers were assayed, the Enroll-HD investigators would have to classify this aspect of the study as interventional because these marker assays are not required for diagnostic assessment. Determination of the number of CAG repeats provides the researchers and patients with information that may assist with diagnostic and clinical management of HD. If data on inflammatory markers had been available, a more accurate test of the potential association between neuroinflammation and HD would have been possible. Given the available data, the best measure of inflammation was the indication of an inflammatory comorbidity in the dataset. However, both available inflammatory diseases, arthritis and MS, are poor proxies for inflammation in the brain because they primarily result from inflammation of the joints or nerves.

Another limitation is that comorbidities, especially arthritis, were underreported in patients with HD. These individuals may have a high pain tolerance and the inflammation of their joints may not have seemed as important in comparison to symptoms of HD. In addition, comorbidities, such as arthritis, may have been over reported in individuals without HD. They may have been more likely to report their arthritis as a comorbidity since they don’t have to manage and constantly report signs and symptoms associated with HD progression. Also, an underlying bias with regards to comorbidities may be present due to self-report. An individual might have reported they had arthritis, when it had not been clinically confirmed, or they may have had arthritis, but not reported it. This bias was less likely to occur for those individuals with MS, as it is a disease with apparent signs and symptoms. Furthermore, the number of participants with inflammatory disorders was low and, therefore, power was reduced.

Although the overall cohort was large, most of this population self-identified as Caucasian, thus limiting the applicability of the conclusions. Additionally, as part of the design of the database, multiple family members were included. Family members share genetic, environmental, and behavioral factors, and thus are correlated, but my analysis methods assumed each participant was independent. Statistical methods are available to adjust for familial correlations, but use of these methods was beyond the scope of this Masters essay. However, because my observations regarding alcohol consumption and smoking use were similar to previously established studies, the non-independence of the observations may not have had a large effect.

A potential future direction using the Enroll-HD cohort could entail tracking the progression of HD and incidence of inflammatory disease over time using the longitudinal data that is available and continues to accumulate. It would also be important to determine if these associations were different based on whether an individual manifests symptoms of HD or not. I would also like to do some adjustments to my data analyses, including controlling for variables that may influence outcome of the test. For example, I would want to control for age when testing the differences among HD subgroups for arthritis, given age is a risk factor for arthritis. Additionally, I could look at other measures of disease severity in HD, such as total motor score and total functional capacity, to assess if prevalence of chronic inflammatory diseases differs by severity of symptoms associated with HD.

This analysis indicates the need for an enhanced method to quantify neuroinflammation in HD patients. In the future, it would be ideal to quantify neuroinflammation with inflammatory factors, especially those most prominent in HD. Recently, a sub-study of Enroll-HD, called HDClarity, has begun clinical trials; its aim is to collect the cerebrospinal fluid (CSF) of 600 participants in various stages of HD and 100 healthy controls to evaluate biomarkers that may be associated with the development of new treatments for HD. Researchers plan to establish a “CSF sample repository” for the storage of samples to encourage continued development of potential therapies for HD. In addition, researchers want to quantify the level of the huntingtin protein in the CSF to study the relationship between this protein’s level and disease severity [113]. I think the CSF of HD patients could be also be taken and analyzed for the quantification of neuroinflammation. This could be an additional component to HDClarity that is added in the future. The goal would be to obtain a better estimate of neuroinflammation in HD patients, which could lead to a better understanding of what inflammatory factors are present and which factors may become present over time, throughout HD progression. If successful, this new understanding of inflammatory biomarkers in HD could be used to predict disease onset and progression of HD, which might allow for improved therapeutics directed at the neuroinflammation piece of HD, Potentially, HD could be controlled further in terms of age of onset, progression and severity, which would give a brighter outcome to HD patients in light of this chronic, debilitating disease.

* + - * 1. **: SUPPLEMENTAL TABLES**

Table 14. Variables pulled from Enroll-HD dataset, but not used in this data analysis.

|  |  |
| --- | --- |
| **Variable:** | **Description:** |
| hdid | Huntington’s disease Identification Number of each participant |
| baseline | Baseline visit for each participant |
| ytd | Years to diagnosis:  y= exp(α+ (β\* x CAPs))  α= 4.4196  β\*= -2.8102  Note: continuous variable |
| tms | Total Motor Score  Note: continuous variable |
| tfc | Total Functional Capacity  Note: continuous variable |
| dcl | Diagnostic Confidence Level: assigned to each participant mainly based on their motor assessment  0= no signs or symptoms of HD  1= clinically at risk for HD  2= clinically prodromal HD  3 or 4= manifest HD, note: 3 or 4 assigned based on severity of HD symptoms  Note: categorical variable |

Table 15. Supplemental population characteristics table specific to HD variables.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Characteristic | Category of participant | | | |
| Manifest HD  (n=4,752) | Pre-manifest HD (n=1,862) | Genotype negative (n=1,089) | Family control  n=1,011) |
| Mean(SD) % | Mean(SD) % | Mean(SD) % | Mean(SD) % |
| YTD | 3.64 (2.92) | 11.09 (6.91) | 13725.1 (37842) | 32096 (60568) |
| TMS | 39.66 (22.25) | 3.55 (5.27) | 1.98 (4.0) | 1.62 (2.90) |
| TFC | 7.80 (3.71) | 12.65 (1.07) | 12.87 (0.74) | 12.86 (0.60) |
| DCL |  |  |  |  |
| 0 | 0.3 | 52 | 76 | 85 |
| 1 | 0.7 | 28 | 21 | 14 |
| 2 | 1.2 | 12 | 2.2 | 0.3 |
| 3 | 2.2 | 6 | 1 | 0 |
| 4 | 96 | 1.2 | 0.5 | 0.2 |

Table . Full results from chi-square test of sex for four HD subgroups in Caucasians.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **HD category** | | | | |
| **Sex** | **Manifest HD** | **Pre-manifest HD** | **Genotype negative** | **Family controls** | **Total** |
| **Male** | 2,219 | 682 | 317 | 411 | 3,629 |
| **Female** | 2,254 | 1,062 | 635 | 509 | 4,460 |
| **Total** | 4,473 | 1,744 | 952 | 920 | 8,089 |
| **Pearson X23 =** 115.58 | | | | **P<** 0.001 | |

Table 17. Subset analyses, full results from chi-square test of sex for manifest HD versus pre-manifest HD in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Sex** | **Manifest HD** | **Pre-manifest HD** | **Total** |
| **Male** | 2,219 | 682 | 2,901 |
| **Female** | 2,254 | 1,062 | 3,316 |
| **Total** | 4,473 | 1,744 | 6,217 |
| **Pearson X21** = 55.62 | | **P<** 0.001 | |

Table . Subset analyses, full results from chi-square test of sex for genotype negative versus family controls in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Sex** | **Genotype negative** | **Family controls** | **Total** |
| **Male** | 317 | 411 | 728 |
| **Female** | 635 | 509 | 1,144 |
| **Total** | 952 | 920 | 1,872 |
| **Pearson X21** = 25.48 | | **P<** 0.001 | |

Table . Full results from chi-square test of alcohol consumption for four HD subgroups in Caucasians.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **HD category** | | | | |
| **Alcohol consumption** | **Manifest HD** | **Pre-manifest HD** | **Genotype negative** | **Family controls** | **Total** |
| **No** | 2,777 | 696 | 446 | 425 | 4,344 |
| **Yes** | 1,694 | 1,044 | 504 | 493 | 3,735 |
| **Total** | 4,473 | 1,744 | 952 | 920 | 8,089 |
| **Pearson X25 =** 301. 28 | | | | **P<** 0.001 | |

Table 20. Subset analyses, full results from chi-square test of alcohol consumption for manifest HD versus pre-manifest HD in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Alcohol consumption** | **Manifest HD** | **Pre-manifest HD** | **Total** |
| **No** | 2,777 | 696 | 3,473 |
| **Yes** | 1,694 | 1,044 | 2,738 |
| **Total** | 4,473 | 1,744 | 6,217 |
| **Pearson X21** = 252.67 | | **P<** 0.001 | |

Table 21. Subset analyses, full results from chi-square test of alcohol consumption for genotype negative versus family controls in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Alcohol consumption** | **Genotype negative** | **Family controls** | **Total** |
| **No** | 446 | 425 | 871 |
| **Yes** | 504 | 493 | 997 |
| **Total** | 952 | 920 | 1,872 |
| **Pearson X21** = 0.0807 | | **P=** 0.960 | |

Table . Full results from chi-square test of smoking use for four HD subgroups in Caucasians.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **HD category** | | | | |
| **Smoking use** | **Manifest HD** | **Pre-manifest HD** | **Genotype negative** | **Family controls** | **Total** |
| **No** | 3,267 | 1,297 | 762 | 749 | 6,075 |
| **Yes** | 1,205 | 445 | 189 | 170 | 2,009 |
| **Total** | 4,473 | 1,744 | 952 | 920 | 8,089 |
| **Pearson X25 =** 45.95 | | | | **P<** 0.001 | |

Table 23. Subset analyses, full results from chi-square test of smoking use for manifest HD versus pre-manifest HD in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Smoking use** | **Manifest HD** | **Pre-manifest HD** | **Total** |
| **No** | 3,267 | 1,297 | 4,564 |
| **Yes** | 1,205 | 445 | 1,650 |
| **Total** | 4,473 | 1,744 | 6,217 |
| **Pearson X21** = 3.48 | | **P=** 0.176 | |

Table 24. Subset analyses, full results from chi-square test of smoking use for genotype negative versus family controls in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Smoking use** | **Genotype negative** | **Family controls** | **Total** |
| **No** | 762 | 749 | 1,511 |
| **Yes** | 189 | 170 | 359 |
| **Total** | 952 | 920 | 1,872 |
| **Pearson X21** = 0.5706 | | **P=** 0.752 | |

Table 25. Full results from chi-square test of inflammatory disease (yes/no) for four HD subgroups in Caucasians.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **HD category** | | | | |
| **Inflammatory disease** | **Manifest HD** | **Pre-manifest HD** | **Genotype negative** | **Family controls** | **Total** |
| **No** | 4,295 | 1,669 | 908 | 851 | 7,723 |
| **Yes** | 178 | 75 | 44 | 69 | 366 |
| **Total** | 4,473 | 1,744 | 952 | 920 | 8,089 |
| **Pearson X23 =** 22.15 | | | | **P<** 0.001 | |

Table . Subset analyses, full results from chi-square test of inflammatory disease (yes/no) for manifest HD versus pre-manifest HD in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Inflammatory disease** | **Manifest HD** | **Pre-manifest HD** | **Total** |
| **No** | 4,295 | 1,669 | 5,964 |
| **Yes** | 178 | 75 | 253 |
| **Total** | 4,473 | 1,744 | 6,217 |
| **Pearson X21** = 0.3312 | | **P=** 0.565 | |

Table 27. Subset analyses, full results from chi-square test of inflammatory disease (yes/no) for genotype negative versus family controls in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Inflammatory disease** | **Genotype negative** | **Family controls** | **Total** |
| **No** | 908 | 851 | 1,759 |
| **Yes** | 44 | 69 | 113 |
| **Total** | 952 | 920 | 1,872 |
| **Pearson X21** = 6.8330 | | **P=** 0.009 | |

Table 28. Full results from chi-square test of arthritis (yes/no) for four HD subgroups in Caucasians.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **HD category** | | | | |
| **Inflammatory disease** | **Manifest HD** | **Pre-manifest HD** | **Genotype negative** | **Family controls** | **Total** |
| **None** | 4,295 | 1,669 | 908 | 851 | 7,723 |
| **Arthritis** | 169 | 68 | 40 | 68 | 345 |
| **Total** | 4,464 | 1,737 | 948 | 919 | 8,068 |
| **Pearson X23 =** 25.08 | | | | **P<** 0.001 | |

Table 29. Subset analyses, full results from chi-square test of arthritis (yes/no) for manifest HD versus pre-manifest HD in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Inflammatory disease** | **Manifest HD** | **Pre-manifest HD** | **Total** |
| **None** | 4,295 | 1,669 | 5,964 |
| **Arthritis** | 169 | 68 | 237 |
| **Total** | 4,464 | 1,737 | 6,201 |
| **Pearson X22** = 0.0566 | | **P=** 0.812 | |

Table 30. Subset analyses, full results from chi-square test of arthritis (yes/no) for genotype negative versus family controls in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Inflammatory disease** | **Genotype negative** | **Family controls** | **Total** |
| **None** | 908 | 851 | 1,759 |
| **Arthritis** | 40 | 68 | 108 |
| **Total** | 948 | 919 | 1,867 |
| **Pearson X22** = 8.66 | | **P=** 0.003 | |

Table 31. Full results from chi-square test of MS (yes/no) for four HD subgroups in Caucasians.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **HD category** | | | | |
| **Inflammatory disease** | **Manifest HD** | **Pre-manifest HD** | **Genotype negative** | **Family controls** | **Total** |
| **None** | 4,295 | 1,669 | 908 | 851 | 7,723 |
| **MS** | 9 | 7 | 4 | 1 | 21 |
| **Total** | 4,473 | 1,744 | 952 | 920 | 8,089 |
| **Pearson X23 =** 3.63 | | | | **P=**0.304 | |

Table 32. Subset analyses, full results from chi-square test of MS (yes/no) for manifest HD versus pre-manifest HD in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Inflammatory disease** | **Manifest HD** | **Pre-manifest HD** | **Total** |
| **None** | 4,295 | 1,669 | 5,964 |
| **MS** | 9 | 7 | 16 |
| **Total** | 4,304 | 1,676 | 5,980 |
| **Pearson X22** = 1.97 | | **P=** 0.161 | |

Table . Subset analyses, full results from chi-square test of MS (yes/no) for genotype negative versus family controls in Caucasians.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **HD category** | | |
| **Inflammatory disease** | **Genotype negative** | **Family controls** | **Total** |
| **None** | 908 | 851 | 1,759 |
| **MS** | 4 | 1 | 5 |
| **Total** | 912 | 852 | 1,764 |
| **Pearson X22** = 2.0151 | | **P=** 0.365 | |

* + - * 1. **: INSTITUTIONAL REVIEW BOARD**

university of pittsburgh irb exempt approved



georgetown university proof of M.holliday on irb at time of access to enroll-hd dataset



huntington’s disease care, education and research center director’s approval for use of dataset



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